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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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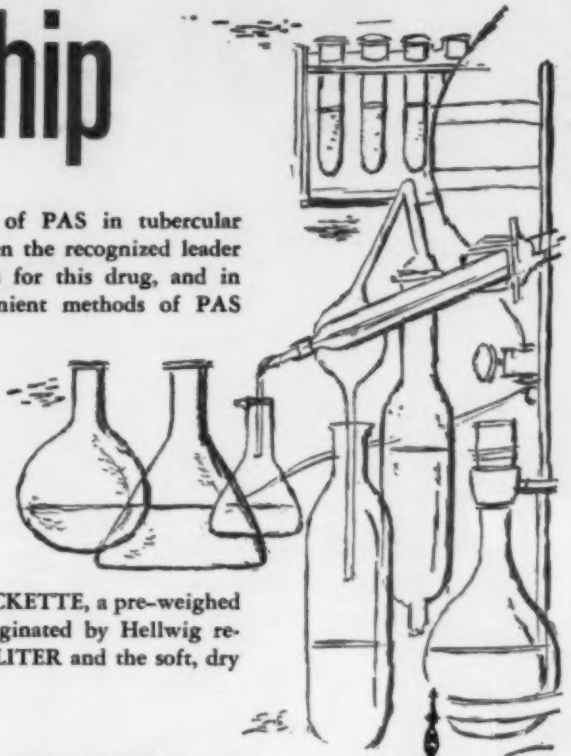

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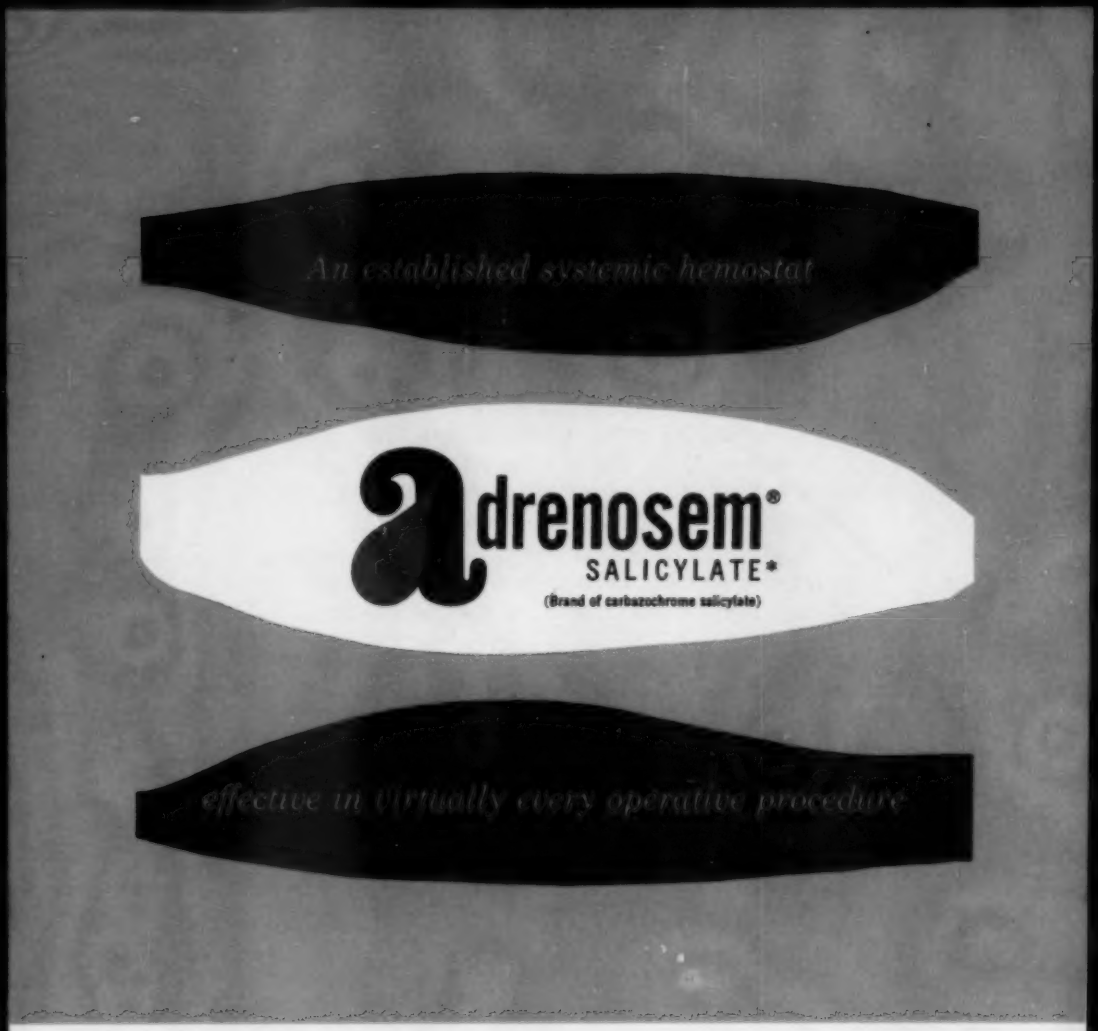

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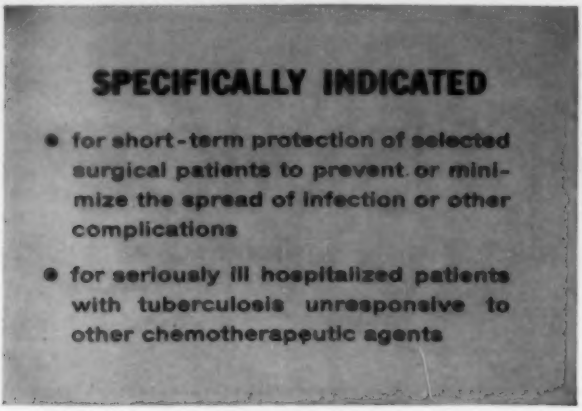
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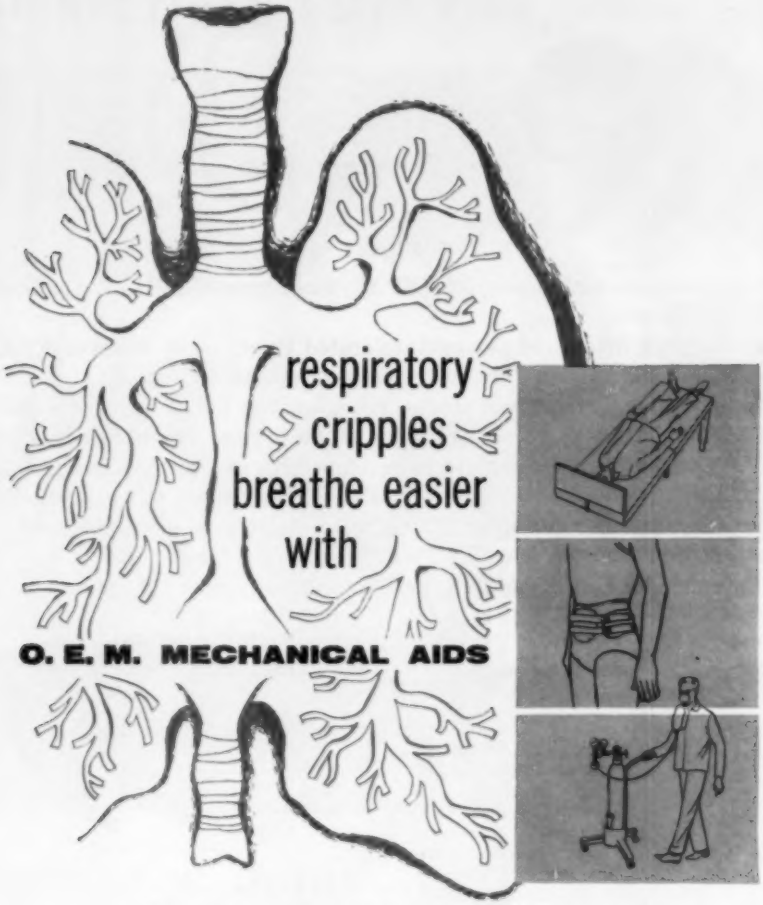

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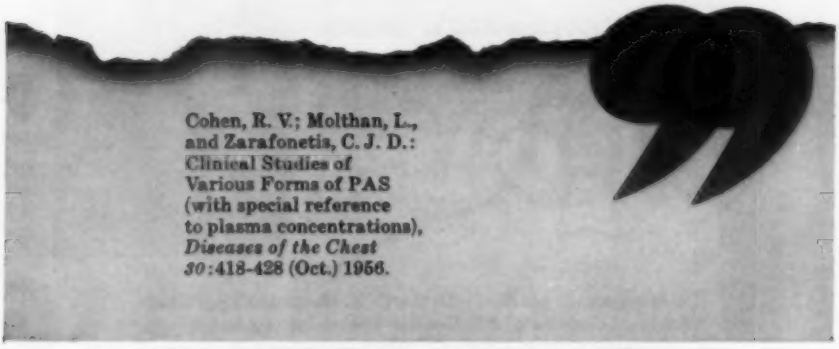

Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* *30*:418-428 (Oct.) 1956.

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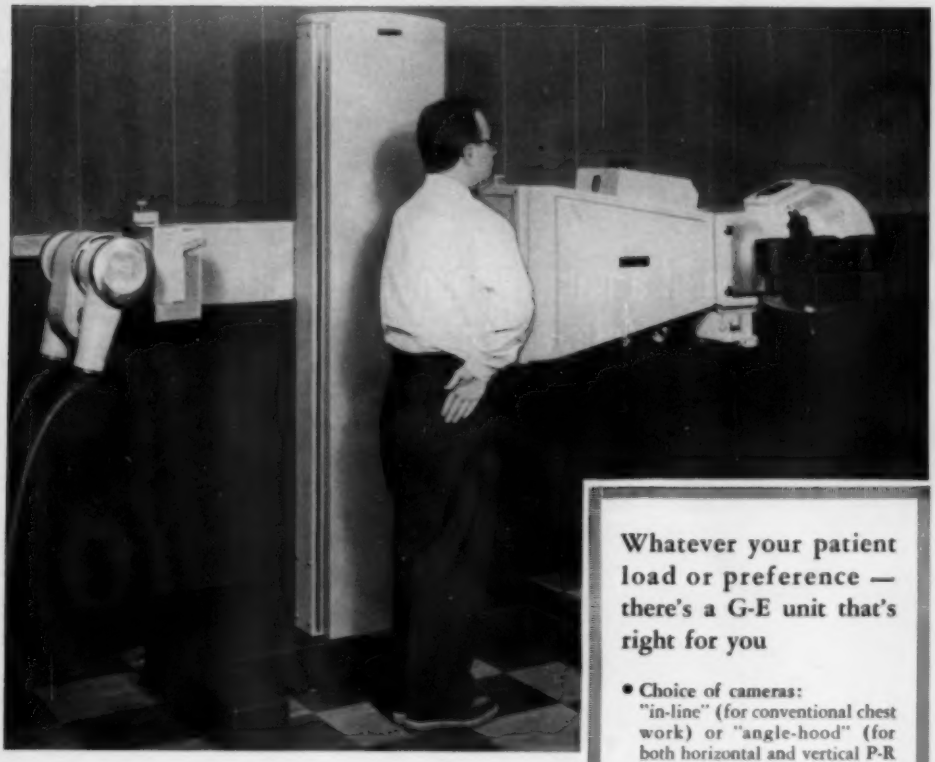

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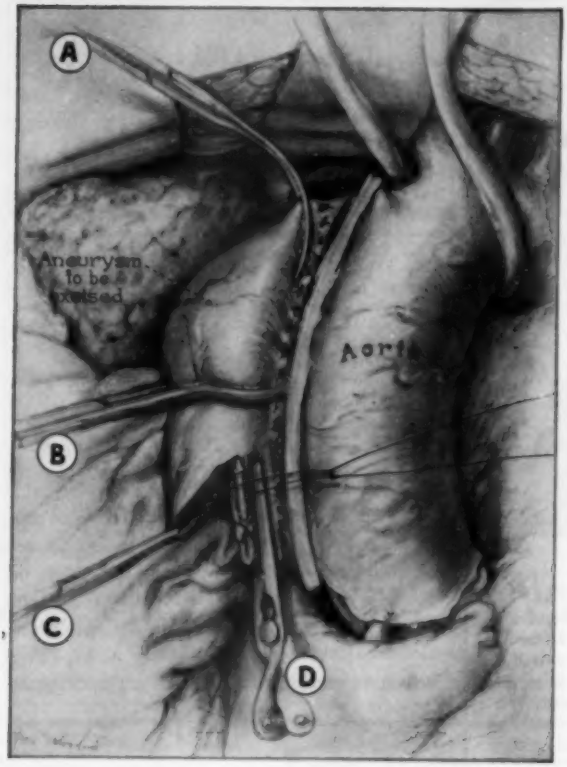

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The Editor wishes to take this opportunity to express his appreciation to Dr. James G. Hirsch, Dr. Kirby S. Howlett, Jr., Dr. Ralph Tompsett, and Dr. William B. Tucker for their high quality of performance and diligent service as members of the Editorial Board.

New members of the Board as of July 1, 1957, are Dr. William R. Barclay, Dr. Aaron D. Chaves, Dr. Leon H. Schmidt, and Dr. Robert L. Yeager, Jr, who have accepted three-year appointments.

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 76 *July, 1957* NUMBER 1

THE USE OF PARENTERALLY ADMINISTERED PANCREATIC DESOXYRIBONUCLEASE AS AN ADJUNCT IN THE TREATMENT OF PULMONARY ABSCESSES[1,2]

JOHN H. AYVAZIAN, ALAN J. JOHNSON, AND WILLIAM S. TILLETT

(Received for publication October 8, 1956)

INTRODUCTION

Prior to the development and use of antimicrobial therapy, abscess of the lung was a serious disease carrying a long period of morbidity and a high mortality. In 1936, Allen and Blackman (1) published a review of 2,114 cases and reported a case mortality of more than 34 per cent, regardless of the method of treatment.

Penicillin and the broader spectrum antimicrobials have radically improved the prognosis for this disease and have reduced the mortality to approximately 5 per cent. Reports (2, 3) on the treatment of pulmonary abscess today stress the excellent response to chemotherapy and the relative rarity of surgical intervention.

Despite this great improvement in the therapy of lung abscess, its period of morbidity often remains long, being measured in months. Moreover, treatment failures still occur which require surgical intervention with its associated complications and mortality.

The rationale for the use of parenterally administered pancreatic desoxyribonuclease as an adjunct to antimicrobial therapy of pulmonary abscess was based upon a number of earlier findings.

In 1948 Sherry, Tillett, and Christensen (4) reported that desoxyribonucleoprotein made up 30 to 70 per cent of the dried sediment of purulent pleural exudates and this desoxyribonucleoprotein accounted for most of the viscosity of the fluid. Following this, Bunting (5) demonstrated by special staining techniques that desoxyribonucleic acid appeared in the interstitium of necrotic or inflammatory tissue with highest concentrations around dead or degenerating leukocytes and parenchymal cells.

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In 1954, Johnson, Goger, and Tillett (6) demonstrated that crystalline pancreatic desoxyribonuclease (referred to as PD) could be given intravenously without observable clinical toxicity and traced its subsequent diffusion into various body exudates and transudates including sputum.

Our consensus, based on these multiple observations, is that desoxyribonucleic acid constitutes a significant proportion of purulent exudate and that this acid originates with inflammation and necrosis, appearing in the interstitium of tissue as it is liberated from the nuclei of degenerating inflammatory and parenchymal cells.

It seemed reasonable to assume that, if depolymerization of the desoxyribonucleic acid within the interstitium of the surrounding tissue and in the abscess proper could be achieved by the parenteral administration of pancreatic desoxyribonuclease, the absorption and drainage of the exudate would be facilitated and healing accelerated.

This report will present the method and results of a study of 7 patients with acute or chronic pulmonary abscesses treated with PD and antimicrobial therapy. The chief objective of the study has been to correlate the clinical course of the patients with quantitative changes in the exudate with respect to the appearance of the parenterally administered PD at the site of disease and the changes in desoxyribonucleic acid effected by the specific enzyme. In view of the difficulty of interpreting clinical changes in the patients as being directly referable to the therapeutic measures employed, the effort has been made to correlate clinical improvement with the results of local measurable enzymatic action. Consequently, both the clinical courses of the patients and the laboratory data of enzymatic activity are described in detail in this report.

Materials and Methods

Methods of administration of pancreatic desoxyribonuclease

Johnson, Goger, and Tillett (6) assayed serial samples of serum following intravenous injections of one million units of pancreatic desoxyribonuclease and found a rapid fall in concentration of the enzyme from concentrations varying between 18 and 50 units per ml. one hour post injection to 1 unit or less after twelve hours.

Experience indicated that higher concentrations of enzyme were obtained in the sputum if the PD was given in larger doses and maintained at higher levels in the serum for longer periods of time. Intravenous infusions of PD were given for periods up to four hours but were found to be inconvenient.

Intramuscular injections were used to maintain prolonged serum concentrations of PD. Studies on 2 patients who received the enzyme in doses of 0.5 mg. per kg. transmuscularly revealed that moderate enzyme concentrations could be maintained for as long as twenty-four hours, a maximal concentration occurring in three to four hours post injection.

A combination of intravenous and intramuscular administration was used to obtain higher serum concentrations. In 6 adults who received 11 mg. intra-

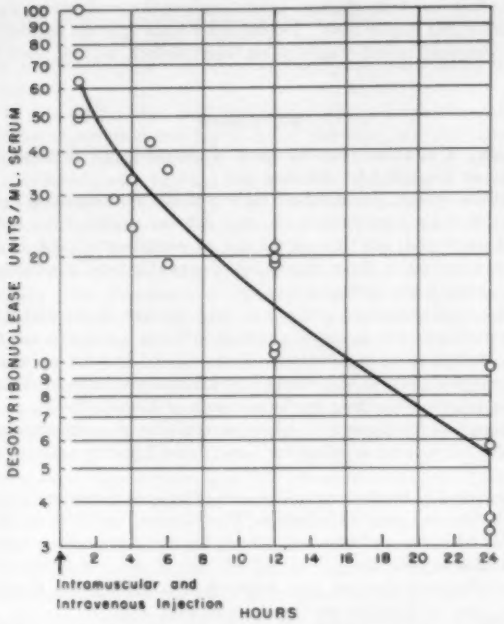

FIG. 1. Semi-log plot of the serum concentrations of pancreatic desoxyribonuclease for 6 patients following the intravenous injection of 1,000,000 units and the intramuscular injection of 2,000,000 units of pancreatic desoxyribonuclease.

venously and 22 mg. intramuscularly, the serum concentrations one hour after the injections ranged from 37 to 102 units per ml. of serum and averaged 63; the twelve-hour concentrations varied from 11 to 21 units and averaged 16, and the twenty-four hour concentrations ranged from 3.3 to 9.6 units and averaged 5.4 (figure 1).

Serial samples were taken from one patient for a forty-eight-hour period after he had received the combined injections at zero and at twenty-four hours. The concentrations in this patient during the second day were consistently higher than those at comparable time intervals during the first day, indicating summation of the enzyme.

METHODS OF STUDY

The patients were treated with all the indicated medical measures which included antimicrobial therapy, bed rest, high caloric diet with vitamin supplements, aerosol bronchodilators, postural drainage, et cetera. Complicating lesions such as neoplasm or tuberculosis were ruled out by appropriate cultures, roentgenograms, bronchoscopic examinations, and Papanicolaou smears. Prior to the administration of pancreatic desoxyribonuclease, the patients were followed through the period of rapid improvement that occurs within the first week or two of antimicrobial therapy until the clinical course and pulmonary lesion had stabilized on the medical regimen. The toxic symptoms initially present and the rapid

improvement that follows chemotherapy would confuse the evaluation of any possible toxic or therapeutic effect PD might have. Twenty-four-hour sputum collections, chest roentgenograms, and complete blood counts were taken before, during, and following enzyme therapy.

MATERIALS

Enzyme substrate: A 0.15 per cent solution of purified calf thymus desoxyribonucleic acid was prepared as described by Johnson (6).

Desoxyribonuclease assay: The method used was the viscosimetric assay developed by McCarty (7), modified by Christensen (8), and further modified by Johnson (6). In the modified method employed, a 0.1 ml. or 0.2 ml. of sample was used as required for optimal enzyme concentrations in the assay which was performed for as long as fifty minutes to obtain a measurable linear fall in viscosity.

The unit of desoxyribonuclease activity is that amount of enzyme which will cause a fall of 1.0 in relative viscosity in ten minutes at 30°C. in a reaction mixture consisting of 2.4 ml. of desoxyribonucleic acid substrate solution and 0.1 or 0.2 ml. of enzyme solution.

Enzyme: The enzyme used in this study was crystalline desoxyribonuclease (PD)[3] prepared from beef pancreas according to the methods of Kunitz (9–11).

The enzyme supplied for *Cases 1* to *5* had an activity of approximately 1,000,000 units per 11 mg., whereas the enzyme supplied for *Cases 6* and *7* had an activity of about 2,000,000 per 11 mg.

A 1 per cent solution of human serum albumin in physiologic saline was used as a solvent and diluent for the enzyme prior to injection. Two hundred and fifty milliliters of this solution were used for intravenous infusions, 10 ml. for the intravenous injections, and 5 ml. for the intramuscular injections.

Feulgen stains: Feulgen staining was done on sputum samples that were smeared on clean glass slides, and air dried as previously described (12).

Sputum viscosity measurements: The viscosity of sputum was measured with a Brookfield Synchro-Lectric Viscosimeter.[4]

Samples

Merthiolate®[5]: Merthiolate was prepared in a stock solution of 1:100 dilution and added as a preservative in all samples to a final dilution of 1:10,000.

Serum: Fresh whole blood was allowed to clot in a clean tube, was centrifuged within one hour, and the separated serum was stored in the frozen state until it was assayed.

Sputum: Sputum was collected in clean glass bottles containing 0.5 ml. of a 1:100 solution of Merthiolate and was stored in the frozen state for at least forty-eight hours. On thawing, the sputum was mixed thoroughly and divided into two parts. The first was filtered through a Buchner funnel with Whatman No. 2 filter paper until 1 ml. of clear filtrate was obtained for desoxyribonuclease assay. The second part was used for the desoxyribonucleic acid assay.

Desoxyribonucleic acid assay: The assay for desoxyribonucleic acid in human sputum is based on a modification of the diphenylamine colorimetric method described by Dische (13).

1. The sputum was prepared for assay by separating it into low polymer, acid-soluble, and high polymer, acid-insoluble fractions. Two milliliters of 10 per cent trichloracetic acid were added to 2 ml. of the homogenized sputum at room temperature and were subsequently centrifuged. The supernatant containing the acid-soluble or low polymer desoxyribonucleic acid was assayed as previously described (13). The sedimented precipitate was reconstituted to its original volume with 5 per cent trichloracetic acid

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[3] Supplied by the Worthington Chemical Co., Freehold, New Jersey.

[4] Brookfield Engineering Laboratories, Sharon, Massachusetts.

[5] Thimerosal, Eli Lilly and Co., Indianapolis, Indiana.

and the residual acid-insoluble or high polymer desoxyribonucleic acid was extracted by heating at 90°C. for fifteen minutes (14). The mixture was then centrifuged and the supernatant assayed for the extracted or "high polymer" desoxyribonucleic acid in the same manner.

2. A method of estimating and correcting for nonspecific interfering color in the diphenylamine reaction was found to be necessary when it was noted that many of the samples with small amounts of desoxyribonucleic acid gave negative results. Recovery experiments with sputum and standard solutions of desoxyribonucleic acid demonstrated incomplete recovery of the desoxyribonucleic acid with some samples of sputum. The amount of desoxyribonucleic acid which the assay failed to recover varied from sample to sample, but was the same for any one particular sputum sample, and was independent of the total amount of standard desoxyribonucleic acid added to the sputum samples. This phenomenon was attributed to the presence of non-desoxyribonucleic acid materials in the sputum that gave interfering color reactions (13). Accordingly, an estimation of the interfering capacity of any given sputum sample was determined by adding 100γ (final concentration) of a standard solution of acid-soluble or low polymer desoxyribonucleic acid to an aliquot of the sputum. Thus, every sputum sample was assayed for desoxyribonucleic acid in three fractions: a low polymer, a high polymer, and a low polymer plus standard. Since there was usually incomplete recovery of the standard when added to sputum, its assay was always less or equal to the sum of the low polymer and the standard assays, respectively, and the difference represented the amount of interference. Consequently, it was possible to correct the low polymer readings for this interference.

The interference was too small to be of significance in the high polymer desoxyribonucleic acid assay, all of which gave strongly positive readings that were consistently reproducible in multiple simultaneous assays of the same specimen.

Observations

Effect of Parenterally Administered Enzyme on Character of Patient's Sputum

In order to determine if the parenterally administered pancreatic desoxyribonuclease would reach the pulmonary lesion and depolymerize the desoxyribonucleic acid present, the sputum specimens from the patients were studied extensively. The samples of sputum were collected daily, before, during, and after PD therapy, and were examined for gross and microscopic physical changes, for desoxyribonuclease activity, and for changes in the quantity and quality of desoxyribonucleic acid.

Physical changes: The physical changes that occurred in the sputum during and following treatment with PD were decrease in volume, decrease in the content of purulent material, increase in the relative content of mucoid material, and increase in gross viscosity. These findings are similar to those observed by Elmes and White (15) who treated a group of patients who had purulent bronchitis with an aerosol of purified noncrystalline pancreatic desoxyribonuclease. These changes occurred normally at a slow rate during the healing phase of chronic lung disease, but it was believed that the parenteral administration of PD accelerated them.

TABLE 1

DESOXYRIBONUCLEASE IN THE SPUTUM OF PATIENTS BEFORE AND DURING THE PARENTERAL ADMINISTRATION OF PANCREATIC DESOXYRIBONUCLEASE

Sputum DNase	Number of Samples	Control	Number of Samples	Treated	p
Concentration, units per ml	30	0.302 ± 0.259	36	0.992 ± 0.882	<0.001
Total, units per 24 hours	29	19.7 ± 27.4	24	50.15 ± 40.11	<0.001

Feulgen stains of smears of control specimens of sputum demonstrated extracellular desoxyribonucleic acid in the form of nuclear fragments, coarse granular particles, and thick, long, well-stained strands representing the various stages of cellular and, more specifically, nuclear degeneration. Concomitant with pancreatic desoxyribonuclease therapy, the extracellular desoxyribonucleic acid did not disappear but did show qualitative changes; the nuclear remnants and course granules were replaced by fine granular particles or were absent entirely; the strands were thinner, shorter, and took the stain less well. After the termination of PD therapy, the qualitative changes tended to revert to the control form, but the concentrations of Fuelgen-positive material on the slides decreased. These changes were similar to the findings of Sherry, Johnson, and Tillett (12) who did Feulgen stains on purulent pleural fluid treated with streptodornase (contained in Varidase[®][6]). The present observations confirmed the findings of Armstrong and White (16*a*) who treated purulent bronchitis with aerosols of purified noncrystalline pancreatic desoxyribonuclease. It should be mentioned here that streptodornase, unlike pancreatic desoxyribonuclease, is a mixture of desoxyribonucleic acid specific enzymes containing nucleotidases and nucleosidases as well as depolymerases (16*b*), but the depolymerase action alone is sufficient to convert desoxyribonucleic acid to non-Feulgen staining fractions.

Sputum desoxyribonuclease—endogenous: Endogenous desoxyribonuclease activity was found in all of 30 control sputum specimens collected from 16 patients (including those with pulmonary abscesses). The enzyme activity ranged from 0.05 to 1.25 units per ml. and averaged 0.30 units per ml. The total daily activity (concentration × 24-hour volume) ranged from 0.6 units to 124 units and averaged 20 units per twenty-four hours.

Sputum desoxyribonuclease post injection of pancreatic desoxyribonuclease: The same 16 subjects were given parenteral PD in individual daily doses ranging from 5.5 to 55 mg. (500,000 to 6,000,000 units depending on the activity of the enzyme) and all but one of them had an increase in the DNase content (concentration and daily total) of their sputum. The only exception was an asthmatic patient who produced mucoid sputum. The 36 samples collected following the injection of PD had DNase activity ranging from 0.20 to 4.4 units per ml. and averaged 0.99 units per ml. The total daily activity ranged from 7 to 182 units per twenty-four hours and averaged 50 units per twenty-four hours.

[6] Streptokinase-streptodornase (Varidase®), Lederle Laboratories, Pearl River, New York.

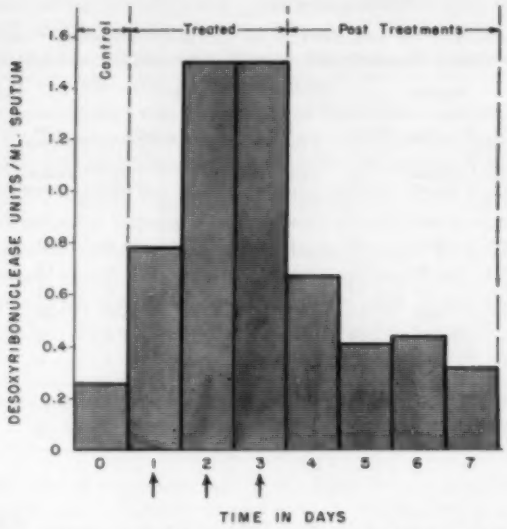


FIG. 2. Average concentration of desoxyribonuclease in the sputum of 6 patients before, during, and after the parenteral administration of pancreatic desoxyribonuclease.

The differences between control and treated sputum are statistically significant with a p of less than 0.001 (table 1).

Six of the patients with pulmonary abscesses received PD for two or more days in succession. The DNase concentrations in the sputum were invariably greater on the second day of treatment than on the first, but were approximately the same as on the third day. After cessation of PD therapy the sputum DNase concentrations fell to control levels in two to four days. This is shown in figure 2.

Sputum desoxyribonucleic acid: For purposes of brevity the data obtained from the patients with pulmonary abscesses have been condensed. The typical case had three days of control observations, three days of PD therapy, and four days of post-treatment follow-up, all consecutively with sputum collections each day. The condensation was achieved by averaging, for each case, the data appropriate to each period, control, treatment, and post-treatment, and tabulating the result under the same heading in table 2.

Analysis of the data, summarized in table 2, revealed a qualitative and quantitative change in the sputum DNA concomitant with and following the parenteral administration of PD.

The qualitative change was an increase in the low polymer fraction in the treated sputum as compared with the controls. In *Cases 1*, *2*, and *5* an absolute increase and, in *Case 6*, a relative increase in the low polymer fraction were demonstrated; whereas, in *Case 3*, in this fraction it was relatively the same and in *Case 7* it was less. (These discrepancies will be discussed further). Thus the average low polymer concentration of the treated sputum for the group was higher than the group average for the control sputum, and this increase is em-

TABLE 2

DESOXYRIBONUCLEIC ACID IN THE SPUTUM OF PATIENTS BEFORE, DURING, AND AFTER THE PARENTERAL ADMINISTRATION OF PANCREATIC DESOXYRIBONUCLEASE

Cases	Control			Treated			Post Treatment		
	Low Polymer	Total DNA*		Low Polymer	Total DNA		Low Polymer	Total DNA	
	Concentration	Concentration	Mg. 24 Hour	Concentration	Concentration	Mg. 24 Hour	Concentration	Concentration	Mg. 24 Hour
	γ/ml.	γ/ml.		γ/ml.	γ/ml.		γ/ml.	γ/ml.	
1	108	292	40	158	327	45.0	25	271	21.0
2	8	1,136	177	64	1,130	122.0	12	906	58.0
3	77	718	79	31	309	21.0	22	173	3.5
5†	31	259	15	71	328	26.0	45	254	9.8
6	25	1,032	11	11	272	2.9	0	160	1.1
7	19	816	15	0	286	1.7	0	288	0.9
Group average..	45	709	56	56	442	36.0	17	342	16.0
Per cent low polymer‡.....	6.3			13			5.0		

Each figure is an average value.

* DNA-desoxyribonucleic acid.

† The data for *Case 4* was excluded as the sputum was grossly contaminated with blood.

‡ Calculated from group averages.

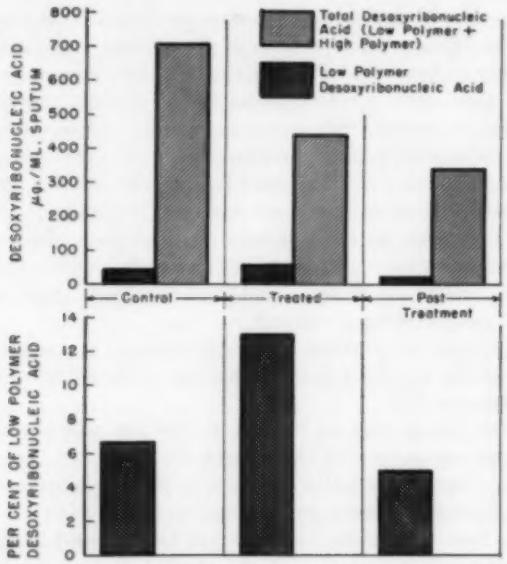


FIG. 3. Effect of the parenteral administration of pancreatic desoxyribonuclease on the depolymerization of desoxyribonucleic acid in the sputum (average of 6 cases).

phasized by calculating the low polymer data as a percentage of the total desoxyribonucleic acid concentration. The significance of this change is enhanced by the reversal of the low polymer fraction in the post-treatment samples to concentrations relatively the same as the controls.

The quantitative change was a decrease in both the concentration and daily production of total desoxyribonucleic acid (low polymer plus high polymer). This decrease began in immediate association with PD therapy in 4 of the 6 cases and was obvious in all 6 cases in the post-treatment period. *Cases 1* and *5* are interesting in that the patients increased their desoxyribonucleic acid output initially, but in both cases this increase was most marked on the first day of pancreatic desoxyribonuclease therapy and by the first post-treatment day their desoxyribonucleic acid output had fallen to a level below that of the controls. This will be discussed further. These changes are summarized in figure 3.

EFFECT OF PARENTERALLY ADMINISTERED ENZYME ON THE COURSE OF LUNG ABSCESS

Case Reports

Case 1. J. J., a 64-year-old Negro male and chronic alcoholic, was admitted to the hospital on October 18, 1954, with a three-day history of fever, shaking chills, cough with "dark, thick" sputum, and a sore mouth. He was acutely ill, with a temperature of 103°F., tachycardia, and tachypnea. Physical examination revealed caries, pyorrhea, an ulcerating stomatitis, and signs of pneumonic consolidation in the left lower lung field.

The results of the laboratory studies were compatible with the presence of an acute bacterial infection. *Neisseria catarrhalis* and *Streptococcus viridans* were cultured consistently from the sputum, and cultures for tubercle bacilli were persistently negative.

Chest films demonstrated a cavity 2.5 cm. in diameter in the lateral basal segment of the left lower lobe with a dense surrounding infiltrate.

The patient was treated with procaine penicillin (1,000,000 units intramuscularly every six hours). His temperature fell from 103 to 101°F.; however, after four days his lesion was unchanged on the roentgenogram and his sputum production was more than 120 ml. a day.

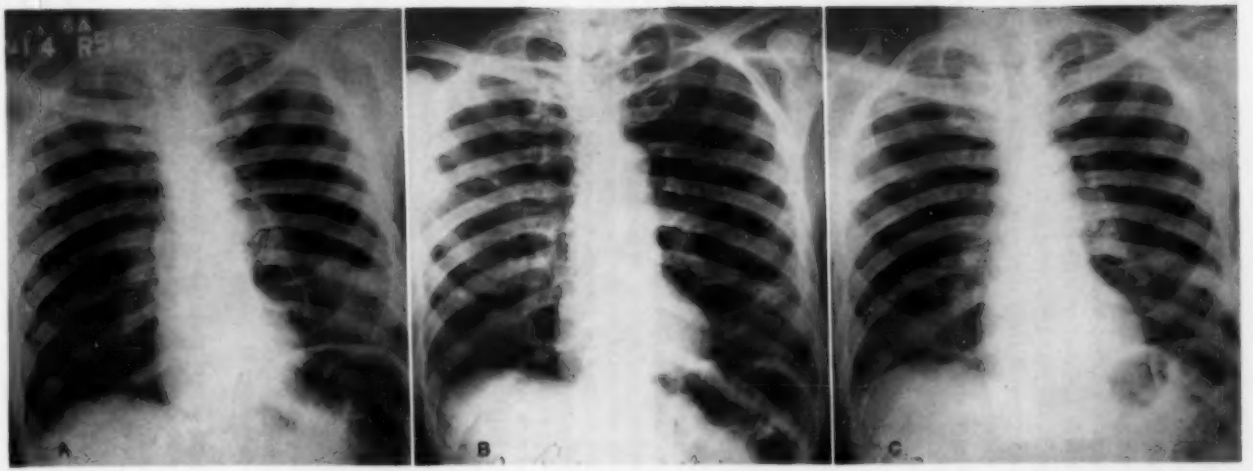
On October 22, 1954, he received 22 mg. of PD (approximately 1,000,000 units per 11 mg.) intravenously in a three-hour infusion and on October 24, he received 55 mg. in a four-hour infusion. Following the first infusion the parenchymal infiltrate surrounding the abscess decreased markedly and a small amount of fluid within the cavity disappeared. Twenty-four hours after the second infusion, the cavity was much reduced in size and the patient's sputum production abruptly decreased as his temperature and leukocyte count fell to normal (figure 4*A*, *B*, and *C*).

During the remainder of the patient's hospital stay his lesion cleared progressively and by November 8, 1954, the roentgenograms showed only a small residual scar. He was discharged asymptomatic on November 20, 1954.

Comment: This case of acute pulmonary abscess demonstrated, by serial roentgenograms, abrupt changes toward healing in immediate association with pancreatic desoxyribonuclease therapy.

Case 2. A. McK., a 43-year-old Negro male, was admitted to the hospital on November 26, 1954, with an eight-month history of cough productive of more than 60 ml. of foul, purulent sputum each day, associated with malaise, weakness, and a 35 pound weight loss.

On admission he had a fever of 101°F. with moderate tachycardia. Examination of his chest revealed diminished excursions of his right thorax with signs of consolidation over the area of his right middle lobe, most marked anteriorly.



Case 1 (J. J.)

FIG. 4*A*. Serial roentgenogram obtained twenty-four hours before administration of pancreatic desoxyribonuclease. *B*. Serial roentgenogram obtained twenty-four hours after administration of pancreatic desoxyribonuclease. *C*. Serial roentgenogram obtained twenty-four hours after the second dose of pancreatic desoxyribonuclease.

Laboratory studies revealed a moderate leukocytosis and anemia. Blood cultures were negative and his sputum contained *E. coli*, *Neisseria catarrhalis*, and *Streptococcus viridans* while being persistently negative for tubercle bacilli.

Despite therapy with procaine penicillin (600,000 units intramuscularly twice daily), the patient continued febrile, and his temperature fluctuated between 100° and 102°F. A roentgenogram confirmed the diagnosis of consolidation of the right middle lobe. Bronchoscopy revealed "large amounts of creamy foul pus exuding from the right upper and middle lobe orifices without evidence of stenosis, dilatation, tumors or ulcerations." Papanicolaou smears were negative for neoplastic cells. On December 5, 1954, chlortetracycline (250 mg. orally four times a day) was added to his therapy because of his lack of response. On December 9, 1954, the patient's temperature rose abruptly to 104°F. and his leukocyte count rose to 18,500 per cu. mm. His sputum production increased to more than 120 ml. daily of green, foul sputum. His temperature remained elevated for two days, then fell to normal by lysis over the next three days. At this time roentgenograms demonstrated an abscess, 3.5 cm. in diameter, in his right middle lobe. On December 16, 1954, the patient was started on a three-day course of pancreatic desoxyribonuclease, receiving 11 mg. intravenously and 22 mg. intramuscularly (approximately 3,000,000 units) each day. Following this therapy the patient's sputum production decreased steadily and his physical findings cleared. His temperature remained normal, and serial roentgenograms showed a progressive decrease in the abscess cavity and its surrounding infiltrate. By January 3, 1955, the cavity was no longer visible and subsequently two series of bronchograms failed to demonstrate any residual cavity.

The patient was discharged asymptomatic on January 18, 1955.

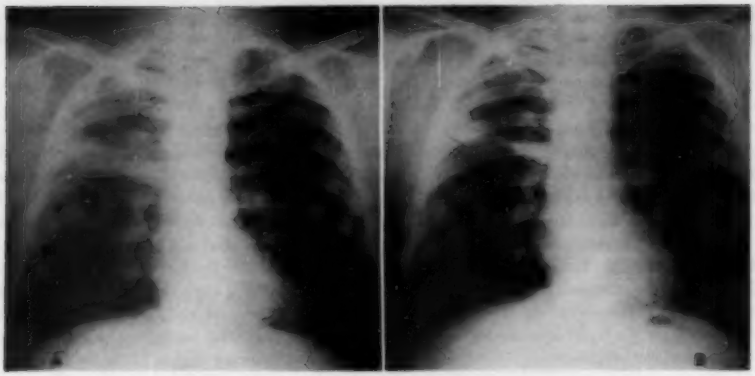
Comment: This patient developed a pulmonary abscess despite two weeks of antimicrobial therapy. Resolution of this lesion began with PD therapy and progressed rapidly to healing.

Case 3. C. M., a 43-year-old white male, was admitted to the hospital on January 3, 1955, with a two-day history of pleuritic right anterior chest pain associated with a hacking cough, producing only small amounts of tenacious "red-brown" sputum and malaise with fever. These symptoms interrupted a prolonged alcoholic bout.

On examination the patient was acutely ill with a temperature of 104°F., tachycardia, tachypnea, and splinting of his right chest. The major physical findings were those of pneumonitis and consolidation of the right upper lobe. He had a normal leukocyte count with only a slight shift to the left. Chest roentgenograms demonstrated complete consolidation of the apical and posterior segments of his right upper lobe. Klebsiella (type *A*) was cultured from blood and sputum.

The patient was started on streptomycin (0.5 gm. intramuscularly twice daily) and chloramphenicol (0.5 gm. orally every four hours). His sputum remained scanty and tenacious and his temperature fell to 101°F. On January 10, 1955, chest roentgenograms revealed "highlights" in the consolidated lobe suggesting necrosis. On January 13, ten days after admission, and on January 14, 1955, he was given 33 mg. of pancreatic desoxyribonuclease each day by the combined intravenous and intramuscular routes. Concomitant with this therapy, the patient's sputum increased to more than 60 ml. a day and he improved clinically, with a decrease in his toxic symptoms. Chest films taken immediately after his course of PD therapy demonstrated complete excavation of the previously consolidated area, leaving a large cavity. Soon thereafter he was transferred to the Surgical Service to be evaluated for possible lobectomy.

During February the patient's abscess cavity remained large with only slight shrinkage in size and a fluctuating fluid level. Three times during the month his sputum production ceased and he developed increased fluid within his cavity associated with abrupt temperature elevations to 105°F., leukocytosis, and acute toxic symptomatology. These episodes were not inhibited by antimicrobials (chloramphenicol and tetracycline) but



Case 3 (C. M.)

FIG. 5*A*. Serial roentgenogram obtained twenty-four hours before the start of pancreatic desoxyribonuclease therapy. *B*. Serial roentgenogram obtained twenty-four hours after the start of pancreatic desoxyribonuclease therapy.

subsided spontaneously with resumption of sputum production and decrease in fluid within the abscess.

On March 1, 1955, four days after the last such episode, he was given a three-day course of PD (11 mg. intravenously and 22 mg. intramuscularly each day). Films taken on the second day of treatment showed an abscess free of detectable fluid for the first time since January 21, 1955 (figure 5*A* and *B*).

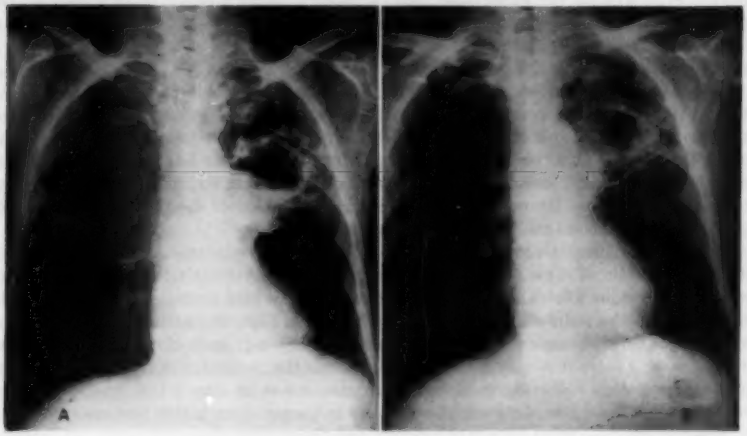
Following this improvement, the patient's sputum production decreased within eight days from a maximum of 150 ml. a day to negligible amounts. The abscess remained free of fluid and shrank rapidly in size. Throughout this period the patient was asymptomatic. The cavity was no longer visualized by routine films after April 12, 1955, and he was discharged without undergoing surgery on June 6, 1955.

Comment: This patient's lung abscess developed secondary to a necrotizing Klebsiella pneumonia. The abscess produced clinical toxicity and failed to drain completely or to heal despite six weeks of antimicrobial therapy. Complete drainage occurred in immediate association with PD therapy and continued until healing had taken place.

Case 4. S. P., a 69-year-old white male, was brought to the hospital on January 25, 1955, with a four-month history of cough and intermittent hemoptysis. Two days before admission he became weak, febrile, and lethargic, followed by confusion and delirium.

On examination he was found to be irrational, irregular periods of lethargy alternating with agitation. He was febrile (102°F.) and had a tachycardia of 160. His major physical signs were marked nuchal rigidity without localizing neurologic signs, and wheezes and rhonchi throughout his chest, most marked on the left.

His clinicopathologic findings were a leukocytosis of 18,500 per cu. mm. with a shift to the left. His cerebrospinal fluid was grossly turbid and contained 8,200 leukocytes per cu. mm., 92 per cent of which were polymorphonuclear leukocytes. The cerebrospinal fluid chemistries were: protein 1,000 mg. per 100 ml.; sugar, less than 10 mg. per 100 ml.; and chlorides, 113 mEq. per liter. Pneumococcus, Type XI, was cultured from the cerebrospinal fluid and cultures of sputum yielded *E. coli* and *Staphylococcus aureus*. Blood cultures were



Case 4 (S. P.)

FIG. 6.4. Serial roentgenogram obtained twenty-four hours before the start of pancreatic desoxyribonuclease therapy. *B*. Serial roentgenogram obtained twenty-four hours after the start of pancreatic desoxyribonuclease therapy.

sterile. An electrocardiogram on admission demonstrated a nodal tachycardia with a ventricular rate of 167. A pulmonary abscess, 5 cm. in diameter, was seen in the left parahilar area on roentgenography. There were linear infiltrates radiating peripherally from the abscess except inferiorly where a dense circumscribed infiltrate, 2 cm. in diameter, impinged on the cavity.

By February 2, 1955, eight days after admission, the patient had responded to therapy with aqueous penicillin (1,000,000 units intramuscularly every two hours), fluid, and electrolytes, and a five-day course of intrathecal pancreatic desoxyribonuclease. He was oriented and cooperative. His cerebrospinal fluid had reverted to normal as did his nodal tachycardia to normal sinus rhythm. However, he continued moderately febrile and his sputum production remained small, less than 10 ml. each day. Roentgenograms obtained during this time showed only slight changes in his pulmonary abscess. Bronchoscopy and Papanicolaou smears revealed no evidence of tumor or obstruction.

On February 2, 1955, the patient was started on a three-day course of PD, receiving 33 mg. each day by the combined intravenous and intramuscular routes. During the five days immediately following the start of this therapy the patient raised appreciable quantities of sputum, approximately 60 to 90 ml. each day. On February 3, 1955, a repeat chest film showed striking changes; his main abscess cavity was free of fluid and smaller in size, and the dense infiltrate below it proved to be a smaller abscess that also had completely excavated (figure 6.4 and *B*). By February 13, 1955, the patient was producing less than 10 ml. of sputum a day, and serial roentgenograms showed that the abscess cavities were consistently free of fluid and shrinking in size.

The patient died on March 2, 1955, twenty-four hours after he developed the signs, symptoms, and electrocardiographic changes of a myocardial infarction. Autopsy was refused.

Comment: This patient with chronic lung abscesses showed an immediate improvement, draining his abscesses within twenty-four hours of the onset of PD therapy.

Case 5. E. S., a 60-year-old white male, upon admission to the hospital on February 25, 1955, gave a history of five days of right anterior pleuritic pain associated with fever, cough, shaking chills, progressive weakness, and malaise.

He was acutely ill with a fever of 105.6°F. and a tachycardia of 120. The positive physical findings were severe pyorrhea, caries, and signs of a pneumonitis in the right midlung field.

Laboratory examination revealed a moderate anemia and a leukocytosis of 15,700 with a shift to the left. His sputum cultured *Staphylococcus aureus*, *Streptococcus viridans*, and *Aerobacter aerogenes*, and was consistently negative for tubercle bacilli. Chest roentgenograms showed a dense "cotton ball" infiltrate, 6 cm. in diameter, in the anterior segment of the right upper lobe. Bronchoscopy revealed inflammation of the right upper lobe bronchus with thick foul pus exuding from the anterior segment.

Despite treatment with procaine penicillin (600,000 units intramuscularly twice daily) and oxytetracycline (0.5 gm. orally four times a day), the patient's temperature remained above 101°F. and, on March 2, 1955, a roentgenogram revealed a cavity, 3 cm. in diameter, in the center of the pulmonary infiltrate. On March 7, 1955, the patient was started on a three-day course of pancreatic desoxyribonuclease, 33 mg. each day, by the combined intravenous and intramuscular routes. At that time the patient was raising about 60 ml. of sputum each day. Following enzyme therapy there was no significant change in sputum production, but there was a sustained reduction in temperature which was associated with a decrease in the fluid level within the cavity and the surrounding infiltrate.

The patient's clinical course tended to stabilize, and a second course of PD was started on March 16, 1955. He received 44 mg. each day for two days by the combined routes (22 mg. intravenously and 22 mg. intramuscularly). On the first day of treatment his sputum production increased to 140 ml., then tapered off within a week to less than 30 ml. a day. His roentgenograms showed a decrease in the size of the cavity and its infiltrate. The patient's subsequent clinical course was one of progressive improvement, with disappearance of the abscess. He was discharged asymptomatic on April 27, 1955.

Comment: This patient received two courses of PD because the improvement noted with the first course had become stabilized. The second course was associated with further changes toward resolution that progressed to complete healing.

Case 6. S. M. was a 70-year-old white male with a history of chest pain, weakness, anorexia, weight loss, and cough which started five weeks before admission to the hospital when he was rendered unconscious in an automobile accident.

On admission on January 27, 1956, he was febrile, lethargic, wasted, and appeared chronically ill. Examination revealed amphoric breath sounds and rales in his right upper chest.

His hemoglobin was 11 gm. per 100 ml. and his leukocyte count was 22,000 per cu. mm.; with a shift to the left. Chest roentgenograms revealed a large abscess, 7 cm. in diameter, with surrounding infiltrate and fluid level in the posterior segment of his right upper lobe. Bronchoscopy was negative for tumor or foreign-body obstruction.

The patient was started on penicillin (aqueous, 300,000 units intramuscularly every four hours) with excellent response. His sputum production was scanty, usually less than 30 ml. of mucopurulent material. By February 7, 1956, his abscess cavity had lost its peripheral infiltrate and fluid level and was reduced in size to 5 cm. in diameter and, although his progress with antimicrobial therapy was excellent, he was given pancreatic desoxyribonuclease in doses of 11 mg. intravenously and 22 mg. intramuscularly each day for three days. There was no significant clinical change that could be directly referred to the enzyme therapy except for a reduction in his sputum volume. His cavity continued to decrease in size gradually. His cough and sputum production ceased by March 1, 1956. He gained weight and strength. By March 29, 1956, his abscess was no longer apparent by routine roentgenographic examination, but tomograms revealed a small 1 cm. cavity in the midst of a fibrotic area. The patient felt well and was discharged on April 21, 1956, for follow-up in the clinic.

Comment: The clinical course of this patient with a chronic lung abscess was one of steady improvement that apparently was not influenced by PD therapy.

Case 7. M. M., a 47-year-old white female, was admitted to the hospital on February 1, 1956, in coma following a suicide attempt with barbiturates.

During her thirty-six hours of coma she developed a fever of 104°F. and a leukocytosis of 35,000 per cu. mm. with a shift to the left. Despite penicillin therapy (procaine, 600,000 units intramuscularly twice daily), she developed signs of a pneumonitis in her left lower lung field and, on regaining consciousness, a cough productive of foul, purulent sputum and pleuritic pain in her left chest. Chest roentgenograms revealed two abscesses in the superior segment of her left lower lobe. The larger cavity was 4 × 2.5 cm., the smaller was 2 cm. in diameter, and both were surrounded by a wide area of infiltration.

Oxytetracycline (250 mg. orally four times daily) was added to her therapy and she improved, becoming afebrile and asymptomatic by February 14, 1956, except for a cough producing 15 to 30 ml. of sputum each day. By February 20, 1956, the patient's abscesses were reduced in size; the largest one was 2 cm. in diameter and the smaller was not clearly visualized in the dense surrounding infiltrate.

On February 20 and 21, she received 11 mg. of pancreatic desoxyribonuclease muscularly each day. Chest roentgenograms, taken on February 22, 1956, showed marked reduction in the infiltrate and the one visible cavity was reduced slightly in size. Sputum production decreased in association with this and, by February 25, 1956, she was producing less than 5 ml. of mucoid sputum a day. On March 9, 1956, the abscesses were no longer evident on chest films, and tomograms taken later showed only residual fibrosis with constriction of the segmental bronchus at the side of the scar and cylindical dilatation of the bronchus distal to it.

The patient's antimicrobial therapy was discontinued on March 18, 1956, and she remained afebrile and asymptomatic until her discharge from the hospital.

Comment: Although this patient's abscesses were responding to antimicrobial therapy, the addition of PD was associated with a marked resolution of the residual lesions, especially the peripheral infiltrate.

INTERPRETATION OF CLINICAL RESULTS

Six of the 7 patients were discharged without clinically apparent pulmonary lesions. In 5 of the cases (*Cases 1, 2, 3, 5,* and *7*), residual scars were present and the patients were considered cured. One patient (*Case 6*) had a 1 cm. residual cavity and is still under observation. Another patient (*Case 4*), whose pulmonary lesion was progressing satisfactorily, died of a myocardial infarction.

Follow-up studies were obtained on 2 patients. One (*Case 1*) was readmitted eight months later for alcoholism, and physical examination and roentgenograms failed to reveal any evidence of pulmonary disease. The other patient (*Case 2*) was readmitted nine months later with generalized peritonitis. He died following a laparotomy, and at autopsy a small nodule of fibrous tissue was found at the site of his pulmonary abscess.

The clinical changes associated with pancreatic desoxyribonucleic acid therapy consisted of a striking decrease in the patient's sputum production and a prompt regression in the pulmonary lesion, as visualized by roentgenograms.

Sputum production invariably decreased in volume and became less purulent and more mucoid in association with PD therapy. The initial change was abrupt and was either a transient increase or a sustained decrease, but in all cases the

sputum production fell to negligible amounts (10 ml. or less) within one to three weeks following PD. The most dramatic example was in *Case 4*, in which the patient did not produce any sputum during a carefully observed three-day control period prior to PD. He began to raise 60 to 90 ml. of sputum daily during the three days of PD therapy; he emptied his abscess cavities, and in the next ten days his sputum output fell to less than 10 ml. a day of mucoid sputum.

The major clinical change noted was in the pulmonary lesion. Serial roentgenograms taken during PD therapy often revealed dramatic improvement, in marked contrast to the gradual, less obvious changes toward healing that occurred during the preceding period of observation.

Six of the 7 patients showed the following changes in immediate association with PD therapy (*see* roentgenograms):

1. Decrease in the area and density of the infiltrate surrounding the abscess
2. Decrease or clearing of the fluid within the cavity when present
3. Decrease in the volume of the cavity

The only exception was in *Case 6* in which the patient improved at an optimal and constant rate before and after PD therapy.

None of the patients in this series had any local or systemic toxic reaction to PD.

The intramuscular injections did not elicit tenderness, erythema, or induration. There were no systemic reactions such as temperature rise, leukocytosis, or allergic manifestations among the 7 patients. However, one patient in a total of more than 150 adults and children given pancreatic desoxyribonuclease parenterally by the present writers developed a severe anaphylactic reaction five minutes after he had received 11 mg. of the enzyme intravenously. He responded to intravenous epinephrine, antihistamines, and aminophylline. This patient had not received PD previously but had a long history of bronchial asthma, and two years earlier he had received daily intravenous infusions of a beef protein hydrolysate[7] for one month. Following this anaphylaxis he was skin tested for sensitivity to protein hydrolysate (Parenamine®) and PD. His skin tests, using a wide range of dilutions, were negative for immediate and delayed reactions to protein hydrolysate, but a strongly positive wheal and erythema reaction developed ten minutes after the intradermal injection of 1γ of PD in 0.1 ml. of saline. Serologic studies failed to reveal a precipitable antibody to protein hydrolysate or a precipitable or neutralizing antibody to PD.

Discussion

The purpose of the present study was to determine: (*1*) whether parenterally administered crystalline pancreatic desoxyribonuclease would reach the site of the lesion in the therapy of pulmonary abscess, and (*2*) whether the local concentration of the enzyme was high enough to change the physical characteristics of the exudate so that its absorption and drainage were accelerated and healing was promoted.

[7] Parenamine, Winthrop-Stearns, Inc.

The significant increase in DNase activity in the sputum from treated patients relative to that from the controls (table 1) implies that PD reached the lesion. However, sputum is a mixture of pus from the lesion, mucus from the glands of the tracheobronchial tree, and saliva. Ideally it would have been best to study the exudate obtained directly from the lesion, but repeated bronchoscopies are impractical and the exudate aspirated at any one time was usually too scanty for assay purposes. To exclude these glandular secretions as the portal of entry of PD into the sputum, serial samples of saliva were obtained from 6 subjects before and after the intravenous injection of PD. There was no significant difference in the DNase concentrations in the sputum before and after the injections. Assays were also done on the predominantly mucoid sputum of 2 patients with asthma and chronic bronchitis. They, too, failed to show an increase in the amount of DNase following intravenous PD. Thus, it is apparent that PD is not actively secreted by the exocrine glands. These and other studies (6) suggest that PD, which has a molecular weight of about 60,000 (17), probably leaves or enters the blood stream by diffusing through the capillary wall. This diffusion is undoutedly favored when capillary permeability is increased by inflammation.

The endogenous DNase activity found in the sputum from control subjects probably originated from sources such as saliva, serum, leukocytes, and certain bacteria and yeasts. All of these materials have been found to have DNase activity (18–23). A statistically significant correlation was found between the DNase and the desoxyribonucleic acid concentrations of the sputum from the control subjects. This correlation may be interpreted as an indication that the DNase activity comes from the same source as the desoxyribonucleic acid, probably leukocytes and possibly bacteria.

Despite the fact that the depolymerization of desoxyribonucleic acid solution by PD decreases its viscosity, the viscosity of the sputum from the treated subjects did not change significantly, and the post-treatment sputum actually increased in viscosity. This was attributable to the relative increase in the amount of mucus in the sputum that occurs with convalescence, as shown previously by Elmes and White (15). Although desoxyribonucleic acid is highly viscous, its effect on the viscosity of sputum is obscured by mucus, the viscosity of which is greater and increases with the inspissation and cooling that follows expectoration.

The decrease in the volume of sputum and its content of purulent material reflects a decrease in the exudation from the lesion and a decrease in the production of mucus. Since exudate probably acts as a topical irritant, stimulating the secretion of the glands of the tracheobronchial tree, a decrease in the amount of mucus would be expected to follow a decrease in exudate. The decrease in sputum volume and purulent material that did occur in association with PD therapy was not due to mechanical obstruction, as it was never associated with the appearance of roentgenographic changes or toxic symptoms that accompany retention of purulent material and increased inflammation.

The changes in the morphology and Feulgen-staining characteristics of the extracellular desoxyribonucleic acid are typical of the action of depolymerases and confirm the findings of previous investigations (12, 16).

The qualitative and quantitative changes in the sputum desoxyribonucleic acid during and after PD therapy also tend to confirm that PD did act on its substrate. Since Kunitz has shown that PD depolymerized desoxyribonucleic acid to acid-soluble oligonucleotides (11), a relative increase in acid-soluble low polymer desoxyribonucleic acid was expected in the sputum following PD therapy. This was obtained in 5 of the 7 cases. The fact that this increase was not seen in 2 cases is probably due to incomplete depolymerization of the desoxyribonucleic acid and increased absorption and diffusion of the soluble depolymerized desoxyribonucleic acid into the body fluids. The method used for the separation and subsequent assay of acid-soluble, low polymer desoxyribonucleic acid recovers only those fragments of the acid with a molecular weight of less than 10,000. As tissue desoxyribonucleic acid has a molecular weight of approximately 3,000,000 (24), an excess of PD is required to depolymerize it to levels at which it can be completely recovered in an acid-soluble phase. Smaller amounts of enzyme tend to cause a slower, less complete depolymerization. This is shown by the Feulgen stains of the sputum of PD-treated patients and by *in vitro* experiments in which marked changes in viscosity of a DNA-DNase solution precede the appearance of acid-soluble desoxyribonucleic acid. In addition, the oligonucleotide end products of depolymerization of desoxyribonucleic acid by PD are dialyzable molecules capable of diffusing from the interstitium into the blood stream as well as into the sputum.

Briefly, the low polymer assay detects only a small part of the depolymerized desoxyribonucleic acid, that which is depolymerized to molecular weights of less than 10,000, and the major part of this is probably absorbed *in situ* and never appears in the sputum.

The daily total desoxyribonucleic acid output tended to decrease with pancreatic desoxyribonuclease therapy, but 2 of the patients (*Cases 1* and *5*) showed the opposite trend initially. A decrease in the total amount of desoxyribonucleic acid in the sputum was thought to be due to increased absorption of the low molecular weight fragments of the acid. An increase was presumed to be due to improved drainage of the exudate (which is mucus free within the abscess and less viscous than sputum) secondary to the decrease in its viscosity. When absorption was increased, the decrease in sputum volume was accompanied by a clinical resolution of the infiltrate surrounding the abscess and a fall in desoxyribonucleic acid (*Cases 2*, *3*, *6*, and *7*). When drainage was markedly improved, the increase in sputum volume was accompanied by a clinical decrease in the fluid level and a rise in desoxyribonucleic acid (*Cases 1*, *4*, and *5*).

The marked decrease in desoxyribonucleic acid output shown by all of the 6 subjects during the post-treatment period reflects the elimination of the residual exudate within the cavity and the decrease in inflammation and exudation within the surrounding tissue.

The therapeutic effects of PD on a suppurative lesion are seen to be primarily due to the increase in absorption and elimination of the products of suppuration, and these may lead secondarily to more efficient antimicrobial activity by the phagocytes and administered antimicrobial drugs.

Of the 7 patients in this series, 3 had chronic lung abscesses (*Cases 3*, *4*, and *6*)

and 4 had acute abscesses (*Cases 1, 2, 5,* and *7*). One patient (*Case 4*) died of unrelated causes and the patients of *Cases 3* and *6* had excellent results relative to the extent and duration of their disease. All of the patients with acute lung abscesses did well. The cavities closed within twenty-four to forty-eight days after the onset of antimicrobial therapy, averaging 33 days for the group. This compares favorably with a group of 37 similar cases reported by Gitten and Mihaly (2) of patients who were treated with antimicrobials alone and closed their abscesses within twenty-two to one-hundred twelve days, averaging more than sixty days for the group.

The antigenic properties of nucleic acid depolymerases have been demonstrated. McCarty (25) and Hazlehurst (26) found neutralizing antibodies to streptococcal DNase in human serum. This antibody was formed following infections with *Streptococcus hemolyticus* or injections of Varidase, a streptococcal extract containing a DNase. McCarty (25) also demonstrated an antibody response in rabbits to purified noncrystalline pancreatic desoxyribonuclease and Johnson (6) found PD was weakly antigenic to humans, producing low titers of a neutralizing antibody in a few of his patients who received PD intravenously. Hence, the occurrence of an allergic reaction was not entirely unexpected and characteristically it occurred in a person with a history of hypersensitivity. By skin testing patients with allergic histories it may be possible to avoid further reactions.

Pancreatic desoxyribonuclease is not required in the routine therapy of most cases of pulmonary abscess, but the present writers believe it has value and can accelerate the processes of healing and shorten the period of morbidity. In certain patients who respond poorly to antimicrobials or fail to establish drainage of their lesions it may be used to advantage and could mean the difference between medical or surgical therapy.

Summary

The rationale for the use of crystalline pancreatic desoxyribonuclease in the therapy of pulmonary abscess is based on the fact that desoxyribonucleic acid constitutes a significant proportion of purulent exudates and is found in the interstitium of inflammatory tissue.

The results presented here demonstrate that, following parenteral administration, pancreatic desoxyribonuclease diffuses into the sputum in significant amounts, probably from the pulmonary lesion, and exerts a depolymerizing effect on its substrate as shown by the qualitative and quantitative changes found in the sputum desoxyribonucleic acid.

The administration of pancreatic desoxyribonuclease was associated with improvement in the patients' clinical course and was confirmed by changes seen in the lesion on the serial roentgenograms.

Sumario

El Uso de la Desoxirribonucleasa Pancreática Administrada Parentéricamente como Coadyuvante en el Tratamiento de los Abscesos Pulmonares

Las razones que abogan el uso de la desoxirribonucleasa pancreática cristalina en la terapéutica del absceso pulmonar se basan en el hecho de que el ácido desoxirribonucleico

constituye una importante proporción de los exudados purulentos y se encuentra en el intersticio del tejido inflamatorio.

Los resultados expuestos ahora demuestran que, a continuación de la administración parentérica, la desoxirribonucleasa pancreática se difunde en el esputo en cantidades considerables, probablemente desde la lesión pulmonar, y ejerce efecto despolimerizante sobre su substrato, según revelan las alteraciones cualitativas y cuantitativas observadas en el ácido desoxirribonucleico del esputo.

La administración de desoxirribonucleasa pancreática se asoció con la mejoría notada en la evolución clínica de los enfermos, confirmando esto las alteraciones observadas en la lesión en las radiografías seriadas.

Resume

L'emploi de la Désoxyribonucléase pancréatique par voie parentérale comme adjuvant au traitement des abcès du poumon

La raison de l'emploi de la désoxyribonucléase pancréatique cristallisée dans le traitement de l'abcès du poumon est basée sur le fait que l'acide désoxyribonucléique constitue une proportion importante des exsudats purulents et qu'il est rencontré dans les espaces interstitiels des tissus inflammatoires.

Les résultats présentés dans cet article démontrent qu'à la suite de l'administration parentérale, la désoxyribonucléase pancréatique diffuse en quantité significative dans l'expectoration, probablement à partir de la lésion pulmonaire, et qu'elle exerce une action de dépolymérisation sur son substrat, comme le montrent les modifications qualitatives et quantitatives de l'acide désoxyribonucléique du crachat.

L'amélioration de l'évolution clinique chez le malade correspondait à l'administration de la désoxyribonucléase pancréatique, ce qui a été confirmé par les modifications des lésions sur les radiogrammes en série.

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THE ACUTE EFFECTS OF SMOKING ON THE MECHANICS OF RESPIRATION IN CHRONIC OBSTRUCTIVE PULMONARY EMPHYSEMA[1]

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Introduction

Although smoking has long been considered to be an aggravating factor in chronic pulmonary emphysema, and has even been assigned a dominant role in its pathogenesis (1), there are few objective data to be found in the medical literature on the acute effects of smoking in this disease. Bickerman and Barach, using measurements of lung volume and ventilatory performance, were unable to find a consistent change following smoking (2). With the development of the intra-esophageal balloon technique for the measurement of changes in intrapleural pressure, a more direct method for quantitating airway resistance has become available. In contrast to the performance measurements in which learning and motivation are of paramount importance, these newer tests can be performed with only the simplest maneuvers once the balloon is properly positioned. Using the balloon technique, Attinger and Segal, at a recent meeting of the National Tuberculosis Association (3), reported an increase in airway resistance following smoking in patients with cardiopulmonary disease. With this method the present writers have studied the acute effects of smoking on the mechanics of respiration in patients with chronic obstructive pulmonary emphysema in normal subjects and in nonemphysematous subjects with respiratory symptoms.

Material and Methods

Thirty-one patients with obstructive pulmonary emphysema, 14 normal subjects, and 5 patients with respiratory complaints but free of emphysema were studied during the past two and one-half years. The diagnosis of emphysema was based on the presence of typical signs, symptoms, and roentgenographic findings, marked diminution of the maximal breathing capacity, increase in the functional residual volume, increase in the ratio of residual volume to total lung capacity above 45 per cent, and an elevated index of intrapulmonary mixing. In the selection of normal subjects, particular attention was given to the absence of signs and symptoms referable to the cardiopulmonary system and the presence of a normal chest film and a normal maximal breathing capacity. Furthermore, in the subjects more than forty-five years of age, anatomic emphysema was excluded on the basis of a normal total lung capacity and its subdivisions.

The determinations using the esophageal balloon were done with the patients in the sitting position. The reproducibility of the method was determined by taking duplicate measurements ten minutes apart, the patients sitting quietly between determinations. The subjects in whom the acute effects of smoking were determined were all habitual

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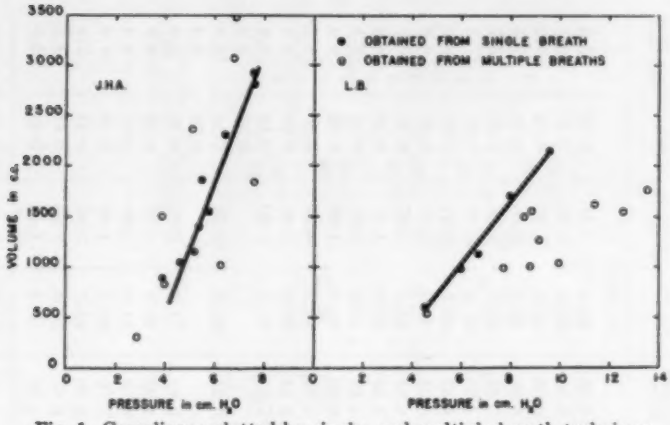

Fig. 1. Compliance plotted by single- and multiple-breath technique

cigarette smokers, but had not smoked for at least six hours prior to the test. They were studied before and immediately after smoking one cigarette. In the study of the effect of simulated smoking on airway resistance, two control measurements were made ten minutes apart. The patient then "puffed" on either an unlit cigarette or on a polyethylene tube of the same dimensions stuffed with cotton so as to be comparable in resistance to a cigarette. A third determination was performed following ten minutes of this. In some cases the patient then smoked a real cigarette and another determination was made; in other instances the simulated smoking was repeated and then actual smoking was done.

Volume flow was measured with a pneumotachometer. The changes in lung volume were determined by continuous electrical integration of the pneumotachometer signal. The pressures were recorded through a polyethylene tube and esophageal balloon as described by Mead and co-workers (4). The pressures were measured with variable inductance manometers, and the output of all instruments was recorded on an oscillograph.

The theory of analyzing the pressure tracings and separating elastic and nonelastic resistance has been previously described by Mead and Whittenberger (5), McIlroy and associates (6), and Dayman (7). The technique was modified so that compliance was computed from one slow, deep inspiration starting from the end-expiratory position. During this breath the air flow falls to zero several times; at these points the pressure and volume are measured and plotted and the compliance computed from the slope of a line connecting these points. By using one slow breath, variations in compliance due to uneven expansion and lag of alveolar pressure behind mouth pressure are minimized (8, 9). Using this single-breath technique, it has been possible to achieve less scatter of points compared with a multiple-breath method (figure 1).

Once the compliance was determined, the pressure necessary to overcome nonelastic resistance was computed in the usual manner (5–7). This value is given as pressure change necessary to produce a volume flow of one liter per second, and will be referred to as airway resistance, although it is realized that it also contains a component of tissue friction. Airway resistance was measured only during inspiration to avoid the effect of positive pressure in expiration narrowing the bronchi and increasing airway resistance (7). Air flow was measured at small volume changes in order to minimize the effect of compliance variations on the computation of airway resistance.

Results

The airway resistance in the patients with emphysema was markedly increased compared with that in the normal group (tables 1 and 2). The mean

TABLE 1

LUNG VOLUMES, INTRAPULMONARY MIXING, COMPLIANCE, AND RESISTANCE IN 25 PATIENTS WITH EMPHYSEMA

Patient	Sex	Age	Height	B.S.A.* (M^2)	Vital Capacity (liters)		Maximal Breathing Capacity (liters/min.)		Total Capacity (liters)	RV/TC	Alveolar N_2, After 7 Minutes O_2	Compliance (liters/cm. H_2O)	Airway Resistance (cm. H_2O/liter/sec.)
					Predicted	Observed	Predicted	Observed					
		years	*inches*							*per cent*			
C. Daw.	M.	59	62	1.60	3.16	2.36	85	60	5.43	56.4	2.37	0.12	13.5
C. A.	M.	43	68	1.70	4.23	1.93	109	41	5.16	62.6	3.90	0.20	9.4
W. Cl.	M.	49	67	1.64	4.07	2.67	100	36	6.03	48.0	4.96	0.14	11.2
D. O.	M.	70	68	1.73	4.12	2.79	87	54	6.68	58.2	3.50	0.38	7.5
L. J.	M.	63	68	1.65	4.17	3.10	88	39	7.66	59.3	2.56	0.20	3.7
O. R.	M.	60	62	1.58	3.43	1.82	87	25	4.35	58.2	3.77	0.09	17.4
J. P.	M.	59	62	1.63	3.32	2.44	91	55	4.83	49.4	2.21	0.18	9.0
R. J.	M.	61	66	1.73	3.88	2.92	95	38	6.38	54.0	4.73	0.37	6.8
H. B.	M.	55	64	1.63	3.62	1.82	95	35	4.94	63.1	5.57	0.21	10.7
F. H.	M.	61	67	1.56	4.01	2.79	85	44	5.68	50.9	4.51	0.17	8.2
T. M.	M.	43	65	1.59	3.80	2.57	102	69	4.52	43.0	2.07	0.19	5.5
G. P.	M.	33	68	1.72	4.01	3.39	116	60	6.80	50.2	1.90	0.15	9.3
L. B.	M.	35	71	2.35	4.31	3.47	160	69	6.00	44.0	4.57	0.26	6.8
H. F.	M.	58	68	1.70	4.07	3.01	90	60	6.54	60.0	6.06	0.29	4.4
C. Dan.	M.	63	66	1.71	3.88	2.32	92	27	7.02	67.0	6.30	0.19	8.0
W. Ca.	M.	58	66	1.56	4.23		88					0.45	5.2
C. St.	M.	59	65	1.48	3.75	2.44	83	35	6.22	62.9	3.58	0.11	16.0
B. C.	M.	70	67	1.73	4.01	2.31	87	17				0.29	8.2
A. C.	M.	67	70	1.87	4.49	3.01	96	60	6.61	54.0	2.92	0.33	3.0
K. H.	M.	40	56	1.75	3.91	1.83	115	35	4.92	62.8	4.52	0.25	6.8
N. W.	M.	66	54	1.56	3.61	2.15	81	49	6.10	64.2	4.20	0.17	3.4
C. Sm.	M.	58	68	1.81	4.17	2.40	102	27	7.76	69.1	4.94	0.45	7.8
L. H.	M.	34	70	1.88	4.90	3.12	129	38	8.20	61.7	3.96	0.27	7.9
R. B.	M.	58	68	1.57	4.17	3.39	91	52	6.97	51.0	3.86	0.67	4.2
J. R.	F.	69	54	1.52	3.16	1.23	59	36	4.64	73.5	4.52	0.25	5.0
Mean		56	65	1.69	3.94	2.55	97	44	6.06	57.7	3.97	0.25	8.0
S. D.....												±0.132	± 3.65

* Body surface area in square meters.

TABLE 2

COMPLIANCE AND RESISTANCE IN 11 NORMAL SUBJECTS

Name	Age	Height	B.S.A.* (M^2)	Vital Capacity (liters)		Maximal Breathing Capacity (liters/min.)		Total Capacity (liters)	RV/TC	Alveolar N_2 after 7 Minutes O_2	Compliance (liters/cm.H_2O)	Airway Resistance (cm. H_2O/liter/sec.)
				Predicted	Observed	Predicted	Observed					
	years	*inches*							*per cent*			
W. C.	53	66	1.58	3.91	3.07	105		4.75	35.4	1.88	0.25	2.0
J. P.	43	68	1.73	4.17	6.99	110	140				0.67	3.7
D. S.	35	68	1.88	4.28	4.05	128	175	5.29	21.4	1.78	0.17	1.6
C. A.	58	63	1.69	3.43	2.66	95	85	3.92	32.1	1.12	0.25	4.3
W. J.	31	69	1.84	4.87	4.50	129	139				0.12	1.8
G. E.	38	67	1.63	4.45	4.18	115	139				0.28	4.2
J. R.	30	65	1.60	4.18	3.91	112	108				0.16	2.0
M. H.	49	65	1.78	3.59	4.76	107	113	6.42	30.0	1.6	0.30	2.6
N. B.	28	69	1.83	4.83	5.92	132	147				0.14	0.8
W. N.	28	71	2.00	4.54	6.08	146	155				0.33	3.5
J. A.	34	72	2.05	5.24	5.97	141	180	8.22	27.4	0.50	0.59	0.7
Mean.....	39	68	1.78	4.32	4.75	120	138				0.30	2.5
S.D.....											±0.18	±1.29

* Body surface area in square meters.

TABLE 3

REPRODUCIBILITY FOR TWO SUCCESSIVE DETERMINATIONS TEN MINUTES APART IN PATIENTS WITH EMPHYSEMA

Name	Compliance (liters/cm. H_2O)			Airway Resistance (cm. H_2O/liter/sec.)		
	No. 1	No. 2	Change	No. 1	No. 2	Change
B. C.	0.33	0.26	−0.07	7.8	8.6	+0.8
A. C.	0.25	0.50	+0.25	2.6	3.5	+0.9
N. W.	0.17	0.17	0.00	3.8	3.0	−0.8
K. H.	0.25	0.25	0.00	6.2	7.4	+1.2
L. H.	0.22	0.36	+0.14	7.6	8.2	+0.6
C. S.	0.33	0.67	+0.34	8.0	7.6	−0.4
R. B.	0.50	1.00	+0.50	4.0	4.2	+0.2
J. R.	0.33	0.20	−0.13	5.0	5.0	0.0
H. B.	0.33	0.25	−0.08	8.2	9.8	+1.6
G. P.	0.14	0.25	+0.11	5.3	5.3	0.0
D. R.				12.6	12.8	+0.2
R. J.	0.25	0.25	0.00	5.6	6.7	+1.1
E. A.	0.29	0.25	−0.04	4.1	3.8	−0.3
A. N.	0.35	0.35	0.00	14.2	16.4	+2.2
L. J.				3.2	3.2	0.0
R. Ja.	0.18	0.18	0.00	11.6	13.0	+1.4
R. Ja.	0.35	0.36	+0.01	10.2	11.2	+1.0
H. F.				11.0	11.0	0.0
Mean Change			+0.07			+0.5

TABLE 4

EFFECTS OF SMOKING ON COMPLIANCE AND AIRWAY RESISTANCE IN EMPHYSEMA

Patient	Compliance (liters/cm. H_2O)			Airway Resistance (cm. H_2O/liter/sec.)		
	Before smoking	After smoking	Change	Before Smoking	After smoking	Change
B. C.	0.26	0.26	0.00	8.6	12.0	+3.4
A. C.	0.50	0.42	−0.08	3.5	5.4	+1.9
N. W.	0.17	0.17	0.00	3.0	4.6	+1.6
K. H.	0.25	0.50	+0.25	7.4	9.2	+1.8
L. H.	0.36	0.22	−0.14	8.2	11.0	+2.8
C. S.	0.67	0.33	−0.34	7.6	10.8	+3.2
R. B.	1.00	0.50	−0.50	4.2	5.1	+0.9
J. R.	0.20	0.20	0.00	5.0	6.5	+1.5
T. M.	0.19	0.10	−0.09	5.5	8.0	+2.5
L. B.	0.26	0.22	−0.04	4.6	12.0	+7.4
G. P.	0.15	0.22	+0.07	9.3	12.6	+3.3
C. D.	0.19	0.19	0.00	8.0	10.0	+2.0
L. C.	0.45	0.38	−0.07	5.2	6.9	+1.7
H. F.	0.29	0.34	+0.05	4.4	4.7	+0.3
C. S.	0.12	0.11	−0.01	16.0	13.5	−2.5
Mean			−0.06			+2.0
S. D.			±0.17			±2.07

TABLE 5

EFFECTS OF SMOKING IN SUBJECTS WITHOUT EMPHYSEMA

Patient	Diagnosis	Age	B.S.A.* (M^2)	Maximal Breathing Capacity (liters/min.)		RV/TC	Alveolar N_2 After 7 Minutes O_2	Compliance (liters/cm. H_2O)			Airway Resistance (cm. H_2O/liter/sec.)		
				Predicted	Observed			Before Smoking	After Smoking	Change	Before Smoking	After Smoking	Change
		years				*per cent*							
V. S.	Bronchial asthma	34	2.00	138	70	20.0		0.45	0.48	+0.03	3.8	3.6	−0.2
R. S.	Bronchial asthma	33	2.01	140	103	33.0	0.56	0.15	0.16	+0.01	8.0	8.0	0.0
S. B.	Bronchial asthma	43	1.82	117	99	26.4	0.92	1.33	0.77	−0.56	2.4	2.4	0.0
J. E.	Chronic bronchitis	44	1.87	119	131	25.4	5.06	0.21	0.21	0.00	5.1	5.1	0.0
J. L.	Chronic bronchitis	41	2.00	130				0.23	0.33	+0.10	5.6	4.2	−1.4
J. A.	Normal	33	2.03	140	161			0.83	0.91	+0.08	0.9	1.2	+0.3
K. H.	Normal	28	1.85	133	121			0.30	0.30	0.00	1.8	2.6	+0.8
N. O.	Normal	24	1.95	144	150			0.22	0.21	−0.01	3.0	3.0	0.0
Y. C.	Normal	29	2.02	144	138			0.28	0.29	+0.01	3.8	4.0	+0.2

* Body surface area in square meters.

value in the emphysematous patients was 8.0 (range 3.0–17.4); for the normal group it was 2.5 (range 0.7–4.3). This difference between the two groups is statistically significant ($p < 0.01$). The mean compliance for the emphysematous patients was 0.25 and for the normal subjects, 0.30; for this measurement no statistically significant difference exists.

The results of the reproducibility study may be seen in table 3. In one subject the change in airway resistance was positive 2.2; in the rest of the subjects the change was positive 1.6 or less, with a mean change of positive 0.5. The mean change in compliance was positive 0.07. In 3 patients (*AC*, *CS*, *RB*) there was a rather marked variation in compliance in the two control runs, but the airway resistance was still reproducible within 1 cm. of water pressure. These studies were of importance in assessing the effects of alterations in compliance on repeated determinations of airway resistance.

In table 4 are presented the results of the acute effects of smoking in 15 patients with emphysema. There was a mean increase in airway resistance of 2.0 following the smoking of one cigarette. This is statistically significant ($p < 0.02$). In 6 of the 15, airway resistance increased more than 2.2, which was equal to the maximal increase in the control studies. All those in whom the airway resistance before smoking overlapped into the normal range increased into the abnormal range with smoking. In only one patient did the airway resistance decrease following smoking. In this study, airway resistance in one patient with early emphysema (*LB*) increased from 4.6 to 12.0 following smoking. In the group as a whole, there was no significant change in compliance.

The acute effects of smoking in 5 subjects with respiratory symptoms but without emphysema and in 4 normal subjects may be seen in table 5. There was no significant change in either compliance or airway resistance in any of these subjects, including those with an elevated airway resistance before smoking.

To explore the possibility that the increase in airway resistance following smoking in patients with emphysema could be due to a downward shift in the mid-position, the effect of smoking on the functional residual volume was studied in 6 patients (table 6). In all but one case there was an increase in the functional residual volume.

TABLE 6

EFFECT OF SMOKING ON THE FUNCTIONAL RESIDUAL VOLUME IN PATIENTS WITH EMPHYSEMA

Patients	Functional Residual Volume (liters)	
	Before Smoking	After Smoking
E. A.*	4.43	4.55
H. F.*	4.65	4.61
R. Ja.	3.22	3.48
A. N	3.58	3.59
L. J.*	4.28	4.69
R. Ja.*	5.88	5.97

* Airway resistance increased with smoking.

TABLE 7

THE EFFECT OF SIMULATED SMOKING ON AIRWAY RESISTANCE IN EMPHYSEMA

Name	Control		Simulated Smoking		Actual Smoking
	I	II	I	II	
D. R.	12.6	12.8	11.4	11.2	8.0
R. Ja.	11.6	13.0	12.6		12.0
R. Ja.	10.2	11.2	9.4	10.0	11.2
A. N.	14.2	16.4	12.6		15.6
J. B.	8.2	9.8	9.4	10.8	10.8
L. J.	3.2	3.7	3.7		4.5
G. P.		5.3	5.3	6.4	7.7
H. F.	11.0	11.0	16.5		20.5
E. A.	3.8	4.1	11.6		20.8
R. Jo.	5.6	6.7	18.0		>18.0
R. Jo.	9.7	11.2	8.8		15.6

The results of the simulated smoking study are presented in table 7. The determinations in the various columns were done in succession at ten-minute intervals. Eleven determinations were made on 9 patients. Two patients (*RJa* and *RJo*) were restudied at two-month intervals. Resistance is given in centimeters of water pressure per liter flow per second as before. There was no significant change in compliance and this is not included. The results are not entirely comparable with those of table 4, as in some cases the balloon was in position for more than one hour. However, in eight of the eleven measurements the airway resistance did increase with smoking. In 3 cases (patients *HF*, *EA*, *RJo*) there was a significant increase with simulated smoking which was invariably accompanied by a further increase when a lighted cigarette was smoked.

DISCUSSION

The measurement of pulmonary compliance and nonelastic resistance by the technique employed here has now undergone extensive investigation in chronic pulmonary emphysema (8, 10). The abnormalities found in the patients and their modification by smoking must be assessed in the light of the pathophysiologic concepts which have developed from such studies.

Emphysema has long been regarded as a disease in which the elastic or retractive forces of lung tissue are seriously reduced by disruption of portions of the lung parenchyma (11). It was therefore not to be expected that several studies (8, 10), in addition to the present one, have been unable to demonstrate such a defect *in vivo*. At first glance it would appear that the compliance measurement should be an adequate index of the integrity of elastic forces in the lung and that its normality, even in advanced emphysema, meant that no true reduction in elastic forces had occurred. Further consideration, however, reveals that the compliance value is determined by many different variables, such as the elasticity of the pleura, surface tension in the alveoli (12), distribution of inspired air (9), and the physical dimensions of the lung (13). Regardless of its patho-

physiologic meaning, the compliance value must be computed if the work of breathing is to be divided into elastic and nonelastic components. In the present study the mean value for compliance is normal in the emphysematous subjects and is not altered by smoking. The technique of analysis was especially designed to minimize the effect of compliance errors on the calculation of nonelastic resistance.

By contrast to the compliance measurement, the estimate of nonelastic resistance was found not only to be increased in the emphysematous subjects but to become further elevated after smoking a single cigarette. Simulated smoking also resulted in an increase in nonelastic resistance in some subjects. However, by contrast with true smoking, in which the changes occurred in 14 of 15 subjects, an increase with simulated smoking occurred in only 3 of 11 subjects. It therefore appears that smoking a lighted cigarette had a reproducible effect on this measurement, whereas "puffing" on an unlit cigarette or on a polyethylene tube was a much less specific stimulus.

Several possible effects could account for this increase in nonelastic resistance with smoking:

Alterations in esophageal tone: Had this occurred, the nonelastic resistance measurements in some of the control subjects should also have been affected.

Reduction in the functional residual capacity: Investigation of this point was believed to be important, since it is well known that the value of nonelastic resistance varies markedly with lung volume, presumably because of variations in the caliber of the airways. However, no evidence for a reduction in functional residual capacity was obtained; in fact, the functional residual capacity increased in size in 5 of 6 subjects tested. This is the change which would be expected following a primary increase in airway resistance.

Changes in tissue friction: There is no way of excluding such a possibility, but at present there is no knowledge which justifies the conclusion that smoking may acutely modify tissue friction.

Reduction in the caliber of the bronchial tree: This conclusion appears to be most in keeping with the known irritating effect of tobacco smoke (14). Such an irritant might be expected to have a relatively greater effect in a bronchial tree which is narrowed. The work of Spain and Kaufman (15) has demonstrated that a reduction in bronchial caliber is actually found in obstructive emphysema. While much of this narrowing is irreversible, the commonly observed therapeutic effect of bronchodilator substance is good evidence that alterations in caliber can occur acutely.

It is concluded, therefore, that in patients with chronic pulmonary emphysema, smoking may be followed promptly by a reduction in the total cross-sectional diameter of the airway. These studies do not tell whether such changes occur because of bronchospasm or bronchial edema, nor do they give information on the length of time the changes may persist.

The demonstration that respiratory mechanics in pulmonary emphysema are rendered more abnormal by smoking could be interpreted as evidence favoring inclusion of smoking among the possible causative agents of this disease. It should be stated, however, that in the present state of knowledge little is known about the cause of emphysema. Moreover, if the effect observed in these studies was one of bronchial irritation, there may be many other substances in the

atmosphere which could produce a similar effect if inhaled in sufficient concentrations.

By contrast, the interpretation of the present study in terms of disability appears to be relatively simple. If disability in pulmonary emphysema is closely related to airway obstruction (16), then a simple and frequently repeated procedure consistently capable of producing an increase in airway obstruction must be presumed to have a harmful effect. Only 2 of the patients (*LB* and *RJo*) actually experienced increased dyspnea after the test cigarette, and in both the increase in nonelastic resistance was of considerable magnitude. Since an increase in dyspnea after smoking is only rarely encountered, whereas an increase in the value for nonelastic resistance is almost invariable, the present writers incline toward the view that all patients with emphysema should be urged to stop smoking irrespective of any apparent relationship between smoking and symptoms.

Summary

Using the intra-esophageal balloon technique, the mechanics of respiration before and after smoking one cigarette were studied in patients with emphysema and compared with the results in a control group of 9 nonemphysematous subjects. An immediate, statistically significant increase in airway resistance was found in 14 of 15 emphysematous patients, whereas no such changes were observed in the control group. There were no significant changes in compliance in either group. The reasons for this increase in airway resistance are discussed, and it is concluded that reduction in the caliber of the bronchial tree is the most plausible explanation. The therapeutic implications of this phenomenon are discussed. While the role of smoking in the development of emphysema is not established, the present study suggests that smoking might be expected to increase respiratory disability in these patients.

Sumario

Los Efectos Agudos del Fumar sobre la Mecánica de la Respiración en el Enfisema Pulmonar Oclusivo Crónico

Con la técnica del globo intraesofágico, se estudió la mecánica de la respiración antes y después de fumar un cigarrillo los enfisematosos, comparando éstos con un grupo testigo de 9 sujetos sin enfisema. En 14 de 15 enfisematosos, se observó en el acto un aumento estadísticamente importante en la resistencia de las vías aéreas, en tanto que no se notaron tales alteraciones en el grupo de testigos. En lo tocante a ejecución, no hubo mayores modificaciones en ninguno de los dos grupos. Discútense las razones de este aumento de la resistencia de las vías aéreas, deduciéndose que la explicación más plausible radica en la reducción del calibre del árbol bronquial. Se discuten los alcances terapéuticos de este fenómeno. Aunque no se ha establecido el papel del fumado en la producción de enfisema, el estudio actual indica que cabría esperar que acrecentara la incapacidad repiratoria en estos enfermos.

Resume

Effets aigus de la fumée sur le mécanisme de la respiration dans l'emphysème pulmonaire obstructif chronique

A l'aide du ballon intraoesophagien, il a été procédé à une étude comparative du mécanisme de la respiration, avant et après que le sujet ait fumé une cigarette, chez sujets

emphysémateux et chez neuf sujets-témoins non-emphysémateux. Une augmentation de la résistance de la voie aérienne, de signification statistique, a été immédiatement décelée chez quatorze des quinze malades emphysémateux; elle n'existait pas dans le groupe de contrôle. Aucune altération significative, en rapport avec ce phénomène, n'a été observée dans l'un ou l'autre groupe. Les raisons de l'accroissement de la résistance de la voie aérienne sont commentées, et les auteurs estiment que la réduction de calibre de l'arbre bronchique est l'explication la plus plausible. Les implications thérapeutiques de ce phénomène sont discutées. Bien que le rôle de la fumée dans le développement de l'emphysème ne soit pas établi, l'étude actuelle suggère que le fait de fumer puisse causer une aggravation des troubles de la respiration chez ces malades.

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AN EVALUATION OF THE ABILITY OF INTERMITTENT POSITIVE PRESSURE BREATHING TO PRODUCE EFFECTIVE HYPERVENTILATION IN SEVERE PULMONARY EMPHYSEMA[1]

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INTRODUCTION

In severe pulmonary emphysema, carbon dioxide retention may occur, interfering with the normal mechanisms of control of respiration. In such a situation some method of increasing effective ventilation in order to reduce the carbon dioxide level toward normal is often desired. An attempt has been made in the present study to determine whether inspiratory intermittent positive pressure breathing can increase the effective ventilation in severe pulmonary emphysema and thereby reduce the carbon dioxide tension, either temporarily or permanently.

Review of the Literature

Pathologic physiology of prolonged carbon dioxide retention: Normally, respiration is controlled by the effect of changes in the carbon dioxide tension upon the respiratory center in the medulla. Scott's (1) report in 1920 of the poor ventilatory response of emphysematous patients with carbon dioxide retention to the inhalation of increasing concentrations of carbon dioxide in air has been amply confirmed (2–6). Fishman and his associates (4, 5), in their studies of the ventilatory response to the inhalation of increased concentrations of carbon dioxide, found that a decreased response was characteristic only of pulmonary emphysema with carbon dioxide retention and that a normal response occurred in emphysema without carbon dioxide retention, metabolic alkalosis and acidosis, and congestive heart failure. They suggested that prolonged elevation of carbon dioxide can permanently injure the respiratory center.

In confirmation of the effect of the prolonged elevation of the carbon dioxide tension on the respiratory center is the work of Schaefer (7), who studied the effect on normal young adults of breathing 3 per cent carbon dioxide continuously for approximately eight days in a climate chamber. The ventilatory effects of prolonged carbon dioxide elevation did not immediately reverse on discontinuing the exposure to carbon dioxide. Several days were required for the bicarbonate to return to normal. Comroe and coworkers (8) found that mental changes often occurred when the carbon dioxide became elevated.

In severe pulmonary emphysema with carbon dioxide retention, oxygen unsaturation is also present. As Motley (9) points out, the factor to be considered in oxygen therapy is administration of oxygen in a high enough concentration to saturate the blood, at the same time permitting adequate alveolar ventilation to wash out carbon dioxide. At the very least, one does not wish to produce hypoventilation with increasing carbon dioxide tension as a result of treating the anoxemia by indiscriminate oxygen inhalation.

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Physiologic effects of mechanical aids to respiration: When oxygen administration produces carbon dioxide retention, both Motley (9) and Barach (10) advise some form of artificial respiration, preferably inspiratory intermittent positive pressure breathing, to wash out carbon dioxide. As Maloney and Whittenberger (11) point out, inspiratory intermittent positive pressure breathing and the body respirator assist ventilation in the same manner, each producing inspiration by creating a pressure difference between the mouth and the outside of the chest wall. They showed that effects of pressure breathing on circulatory dynamics depend on the shape of the intratracheal pressure curve and not on its mode of application. Although most patients can tolerate a negative intra-tank pressure of 15 to 20 cm. of water, caution must be practiced in the presence of unstable circulatory control since circulatory collapse, decreased cardiac output, and shock may be precipitated. Alternating positive and negative pressure does not have as great an effect on the circulatory dynamics as has been reported by Maloney and Handford (12).

Cournand and his co-workers (13) studied the effect of different types of intermittent positive pressure breathing on cardiac output and right ventricular filling pressures by means of cardiac catheterization in man. They distinguished three types of intratracheal pressure curves: (*1*) expiration equal in duration to inspiration and the resting pressure slightly above atmospheric; (*2*) long inspiration and short expiration; and (*3*) initial rapid fall of pressure during expiration, but the total duration of expiration equal to inspiration. It was only when using apparatus producing a type 3 curve that no significant change in cardiac output or right ventricular filling pressure occurred. Segal and Attinger (14) investigated the type of pressure curve produced by the Bennett, Halliburton, Emerson, MSA, and Vent Elaire intermittent positive pressure breathing units. All but the MSA apparatus produced a type 3 mask pressure curve.

Methods

The effect of intermittent positive pressure breathing was studied on male subjects, all of whom, with the exception of the 2 younger subjects with normal lungs, had severe pulmonary emphysema which had reached a fairly stable state after many weeks to months of hospitalization. At the time of examination they were not undergoing any acute exacerbation of their disease. The medications, such as potassium iodide, bronchodilators, and digitalis, were continued unchanged during the period of testing. None of the patients had a clinically significant component of allergic asthma; many had cor pulmonale. Before each series of tests all subjects rested in bed for at least thirty minutes but were not in a fasting state. The oxygen and carbon dioxide content and oxygen capacity were determined by the method of Van Slyke and Neill on blood obtained from an indwelling needle in the femoral or brachial artery. (An oxygen saturation of 97.5 per cent and carbon dioxide content of 50 volumes per cent are normal in our laboratory.) The pH was determined immediately in a closed glass electrode using the Beckman pH meter. (A pH of 7.42 plus-minus 0.02 is considered normal.) The carbon dioxide tension was calculated by using the Henderson-Hasselbalch equation. (A partial pressure of 40 mm. of mercury is normal and a change of 6 mm. of mercury is considered to be significant.)

The minute ventilation was obtained by collecting the expired gas in a Tissot spirometer equipped with a motor-driven kymograph and recording drum. A three-minute expired gas sample was used to calculate the minute ventilation both in the resting state and during the last three minutes of intermittent positive pressure breathing therapy. The gas volumes are reported at BTPS. An arterial blood sample was obtained during the resting state and in the last minute of intermittent positive pressure breathing therapy.

The Emerson Respiration Assistor® was used to provide intermittent positive pressure breathing in all but one instance (*L. H.*, for whom a Bennett apparatus was used.) When an oxygen tank was used to activate the apparatus, a mixture containing 50 to 60 per cent

oxygen was delivered to the mouth. A pressure of 15 cm. of water above atmospheric pressure was used on inspiration. Expiration was at atmospheric pressure. Unless otherwise stated, a 2 ml. volume of isopropylarterenol hydrochloride (Isuprel®) in 1:200 dilution was used in the attached nebulizer, and intermittent positive pressure breathing therapy was given for a period of fifteen minutes.

Voluntary hyperventilation was produced by instructing the patient to breath more than normally but not to attempt to perform a maximal breathing capacity. The patient was permitted to choose his own depth and rate of respiration. A few short practice trials were performed followed by a rest period before the recorded test was done. Hyperventilation was maintained for four to eight minutes, the duration depending on the degree of fatigue produced. The arterial blood sample was obtained during the last minute of voluntary hyperventilation. The entire period of voluntary hyperventilation was recorded on the Tissot, and the average value for the entire period was determined and entered in table 5.

Results

Intermittent positive pressure breathing with oxygen: In table 1 may be seen the ventilatory and arterial blood values before and at the end of fifteen minutes of intermittent positive pressure breathing therapy with oxygen and isopropylarterenol hydrochloride. The subjects studied have been separated into three groups: 2 young men with normal lungs; 7 persons with severe pulmonary emphysema whose carbon dioxide content was less than 60 volumes per cent; 6 persons with severe emphysema and a carbon dioxide content of more than 60 volumes per cent.

The 2 normal males hyperventilated while receiving intermittent positive pressure breathing, increasing their minute ventilation approximately 12 liters. This was accomplished principally by increasing the tidal volume more than twice the resting level with little change in the respiratory rate. The increase in their effective ventilation was substantiated by the drop in the carbon dioxide tension. The arterial blood became supersaturated.

The next group of subjects with severe emphysema and a carbon dioxide content of less than 60 volumes per cent responded in two different ways. The first 3 patients listed increased their ventilation by increasing the tidal volume and the respiratory rate. The carbon dioxide tension fell slightly. The remaining 4 patients did not hyperventilate appreciably, and this is substantiated by the unchanged carbon dioxide tension. Their respiratory rate increased, but the tidal volume showed little change. One patient (*L. H.*) did not have his ventilation measured.

The severely emphysematous subjects with a carbon dioxide content of more than 60 volumes per cent fell even more strikingly into two groups than the previous patients. The first 2 patients (*W. H.* and *J. L.*) hyperventilated during intermittent positive pressure breathing with oxygen. In contrast to those patients without significant carbon dioxide retention, a fall in respiratory rate occurred, the tidal volume increasing. The carbon dioxide tension was reduced. The 4 patients who hypoventilated increased their P_{CO_2} by 6 to 17 mm. of mercury. Ventilatory studies could be done on only 2 of these patients. Their tidal volume remained substantially unchanged but the respiratory rate fell. It should

TABLE 1

EFFECT OF INTERMITTENT POSITIVE PRESSURE BREATHING WITH OXYGEN AND ISOPROPYLARTERENOL HYDROCHLORIDE FOR FIFTEEN MINUTES UPON THE ARTERIAL BLOOD AND VENTILATION

Patient	Oxygen Saturation Per Cent		Carbon Dioxide Content Volume Per Cent		P_{CO_2} (mm. Hg)		pH		Minute Ventilation (L./min.)		Respiratory Rate		Tidal Volume (ml.)		Remarks
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	
Normal Young Males															
FH	98.5	100+	48.6	40.5	38.3	20.7	7.45	7.66	7.34	19.45	16.7	19.7	440	988	
CB	97.5	100+	49.4	43.6	36.4	24.1	7.48	7.62	6.60	18.50	15.0	15.2	570	1,200	
Severe Emphysema but Carbon Dioxide Content Less Than 60 Vol. Per Cent															
TvW	80.0	100+	57.6	55.2	52.0	43.9	7.38	7.43	9.20	18.60	13.0	21.2	708	877	
MB	84.3	100+	56.1	52.8	50.9	43.3	7.37	7.42	9.40	16.90	14.7	18.7	640	905	
CA	81.0	100+	56.0	55.1	48.8	42.8	7.40	7.46	8.83	14.40	17.7	19.0	500	758	
JH	95.6	100+	57.0	55.4	47.2	46.3	7.43	7.43	8.45	11.40	18.4	20.2	460	565	
FP	93.4	100+	52.1	52.7	38.7	34.7	7.46	7.52	9.00	15.85	17.0	27.0	531	587	
FA	89.5	100+	55.1	57.3	40.0	41.8	7.48	7.48	15.10	18.60	36.0	54.0	418	343	
LH	93.4	100+	56.3	57.3	46.2	47.0	7.42	7.42	—	—	—	—	—	—	Used Bennett IPPB* for 3 years
Severe Emphysema but Carbon Dioxide Content More Than 60 Vol. Per Cent															
WH	84.6	100+	62.3	58.8	56.5	49.5	7.39	7.42	8.55	11.85	16.7	12.7	512	931	
JL	64.6	100+	76.1	71.7	61.6	44.8	7.45	7.58	6.25	11.12	19.0	13.7	329	814	
CC	80.0	100+	67.6	71.5	60.6	73.4	7.38	7.30	5.90	4.05	12.3	7.7	480	540	
WF	63.2	100+	76.8	82.2	57.6	70.6	7.45	7.38	—	—	—	—	—	—	
ET	59.8	100+	79.6	85.1	66.4	83.2	7.42	7.34	7.16	4.23	29.4	15.3	244	277	Nasal oxygen and IPPB with oxygen caused coma
HG	88.0	100+	78.6	80.8	65.3	71.8	7.41	7.38	—	—	—	—	—	—	On nasal oxygen continuously (3 L/min.)

* IPPB—intermittent positive pressure breathing.

TABLE 2

EFFECT ON ARTERIAL BLOOD AND VENTILATION OF VARIOUS TYPES OF BREATHING

Patient	Oxygen Saturation, Per Cent			P_{CO_2} (mm. of mercury)			Minute Ventilation (L./min.)			Respiratory Rate			Tidal Volume (ml.)		
	RA	IPPB	O_2	RA	IPPB	O_2	RA	IPPB	O_2	RA	IPPB	O_2	RA	IPPB	O_2
JL	67.9	89.8	99.1	61.2	44.0	70.0	6.8	8.8	4.2	18.7	11.0	19.7	364	800	214
WH	89.0	100+	—	53.0	37.4	—	8.2	13.9	—	17.0	13.2	—	483	1,060	—
TvW	90.4	93.8	97.8	46.1	35.5	49.0	9.4	20.3	9.6	10.8	21.3	13.7	875	965	700
CC	78.7	88.2	—	59.4	58.3	66.9	6.9	4.7	—	15.3	13.0	—	450	360	—
WF	61.6	72.9	94.6	60.7	59.5	66.4	7.0	7.9	5.8	11.6	10.6	12.6	600	750	456
ET	67.8	75.8	96.8	67.7	69.2	74.2	8.2	8.3	6.9	29.6	23.0	29.3	275	357	235

Room air (RA)

IPPB with compressed air and isopropylarterenol hydrochloride (IPPB)

50% oxygen at atmospheric pressure (O_2)

Using IPPB with compressed air, but without isopropylarterenol hydrochloride there was the following fall in P_{CO_2}: *J.L.* = 11.8 mm. of mercury

W.H. = 8.5 mm. of mercury; *T.vW* = 0 mm. of mercury

be noted that all of these patients with carbon dioxide retention had pH values within the normal range at rest.

Intermittent positive pressure breathing with compressed air: Further studies were done on 6 of the patients with severe emphysema, using a tank of compressed air instead of oxygen to activate the intermittent positive pressure breathing unit. Within a minute or two of the completion of approximately fifteen minutes of intermittent positive pressure breathing with compressed air and isopropylarterenol hydrochloride, all but one of the patients were switched to a 50 per cent oxygen mixture at ambient pressure. The gas mixture was inhaled for fifteen minutes more, and then the ventilatory and arterial blood studies were repeated. The results of this study are shown in table 2.

The first 3 patients (*J. L.*, *W. H.*, and *T. vW.*) had hyperventilated during intermittent positive pressure breathing with oxygen. Only one patient (*T. vW.*) belonged in the group with a carbon dioxide content of less than 60 volumes per cent. As shown in table 2, the patients hyperventilated to as great or greater degree with intermittent positive pressure breathing with isopropylarterenol hydrochloride and compressed air as they did with oxygen. Again, they did this principally by increasing their tidal volume. It is interesting that the 2 patients with carbon dioxide retention reduced their respiratory rate, while the one patient (*T. vW.*) with normal carbon dioxide increased his respiratory rate, just as the other patients did when oxygen was used. It was not appreciated until this study was over that the fraction of oxygen in the compressed air tanks used in this hospital varies from 18 to 25 per cent and is usually more than 21 per cent. Therefore, the improved oxygen saturation cannot be entirely attributed to intermittent positive pressure breathing.

The 2 patients (*J. L.* and *T. vW.*) who were switched to 50 per cent oxygen at ambient pressure while still under the influence of isopropylarterenol hydrochloride reacted differently. One (*J. L.*) with carbon dioxide retention hypoventilated. His tidal volume fell and respiratory rate increased to the pretreatment level. The other patient (*T. vW.*), whose carbon dioxide was normal, reduced his ventilation as well as his respiratory rate to the pretreatment level.

Three patients who hyperventilated were tried on another day with intermittent positive pressure breathing with compressed air but not with isopropylarterenol hydrochloride. Two patients (*J. L.* and *W. H.*) hyperventilated slightly; the other did not. It is apparent that in all 3 of these patients the bronchodilator enhanced the hyperventilatory effect of intermittent positive pressure breathing.

The other 3 patients (*C. C.*, *W. F.*, *E. T.*) shown in table 2 had carbon dioxide retention and hypoventilated when given intermittent positive pressure breathing with oxygen and isopropylarterenol hydrochloride. When using intermittent positive pressure breathing with compressed air and isopropylarterenol hydrochloride, there were no significant changes in P_{CO_2} or ventilation. There was slight improvement in the oxygen saturation. Switching to 50 per cent oxygen at ambient pressures, as in the previous group, caused an increase in P_{CO_2} above the resting level and a drop in minute ventilation. However, these hypoventilatory changes were not nearly as marked as when intermittent positive pressure

TABLE 3

EFFECT ON THE ARTERIAL BLOOD OF DAILY USE OF INTERMITTENT POSITIVE PRESSURE BREATHING WITH ISOPROPYLARTERENOL HYDROCHLORIDE

Patient	Days of Treatment Using IPPB* with		Oxygen Saturation Per cent		Carbon Dioxide Content Volume Per Cent		P_{CO_2} (mm. Hg)		pH	
	O_2	Air	B	A	B	A	B	A	B	A
HG	4	—	88.0	84.6	78.6	75.7	65.3	64.4	7.41	7.40
JL	7	—	64.5	71.8	73.8	69.6	63.6	58.6	7.42	7.42
JL	—	4	71.8	59.0	69.6	75.2	58.6	63.9	7.42	7.42

* IPPB—intermittent positive pressure breathing.

breathing with oxygen was used. Perhaps this is partly due to the fact that full oxygen saturation may be attained later than when intermittent positive pressure breathing is used to deliver the oxygen.

It is revealed in these experiments that, whereas intermittent positive pressure breathing with oxygen sometimes produces hypoventilation, this does not occur when compressed air is used. Those subjects who hyperventilate during intermittent positive pressure breathing with oxygen will also hyperventilate when compressed air is used, and the degree of hyperventilation is apt to be greater. Finally, in those patients in whom hyperventilation occurred, it was found that a bronchodilator such as isopropylarterenol hydrochloride increased the effectiveness of the procedure.

The duration of the effects of intermittent positive pressure breathing: Two patients (*H. G.* and *J. L.*) received intermittent positive pressure breathing with oxygen and isopropylarterenol hydrochloride for fifteen minutes three times a day for four to seven days. The arterial blood findings before and after this period of treatment are shown in table 3. There was no significant change in the resting carbon dioxide content or tension in either patient. *J. L.* received intermittent positive pressure breathing therapy for four additional days, compressed air being used without effect on his carbon dioxide retention. The changes in the arterial blood noted in *J. L.* were no greater than those seen in this patient from day to day when he did not receive intermittent positive pressure breathing.

Another indication of the transient nature of the reduction in carbon dioxide produced by this therapy in patients with long-standing carbon dioxide retention are some further studies performed on *J. L.* as shown in table 4. After receiving intermittent positive pressure breathing therapy with oxygen and isopropylarterenol hydrochloride for fifteen minutes, his ventilation was measured for an additional fifteen minutes after such therapy was discontinued, and an arterial blood sample was obtained at the end of that time. In the first three minutes the ventilation fell markedly, but the respiratory rate was unchanged. Then, during the ensuing twelve minutes, the minute ventilation and respiratory rate gradually increased. At the end of fifteen minutes the carbon dioxide content and tension were at the pretreatment level. The same sequence of events occurred when intermittent positive pressure breathing with isopropylarterenol hydrochloride and compressed air was used.

TABLE 4

ARTERIAL BLOOD AND VENTILATORY VALUES BEFORE, AT THE TERMINATION OF, AND FOR FIFTEEN MINUTES AFTER INTERMITTENT POSITIVE PRESSURE BREATHING

Patient	Oxygen Saturation, Per Cent		Carbon Dioxide Content Volume Per Cent		P_{CO_2} (mm. Hg)		pH		Minute Ventilation (L./min.)		Respiratory Rate		Tidal Volume (ml.)		Remarks
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	
JL, using oxygen	71.8	100+	69.6	63.7	58.6	41.2	7.42	7.55	6.15	11.4	12.0	11.7	510	970	Results before and at end of IPPB*
										4.53		12.7		356	Three minutes after IPPB
										4.13		10.0		410	Six minutes after IPPB
										5.30		15.0		353	Nine minutes after IPPB
										7.00		17.7		398	Twelve minutes after IPPB
		85.2		72.4		62.2		7.41		6.42		17.7		366	Fifteen minutes after IPPB
JL, using air	59.0	87.3	75.2	71.9	63.9	45.2	7.42	7.57	7.81	9.19	20.3	12.0	355	765	Results before and at end of IPPB
										4.63		14.3		323	Three minutes after IPPB
										5.00		14.0		357	Six minutes after IPPB
										6.52		18.0		364	Nine minutes after IPPB
										7.54		18.7		403	Twelve minutes after IPPB
		67.5		74.9		58.7		7.46		6.74		20.0		337	Fifteen minutes after IPPB

* IPPB—intermittent positive pressure breathing.

TABLE 5

EFFECT ON ARTERIAL BLOOD AND VENTILATION OF VOLUNTARY HYPERVENTILATION

Patient	Oxygen Saturation Per Cent		P_{CO_2} (mm. Hg)		Minute Ventilation (L/min.)		Respiratory Rate		Tidal Volume (ml.)	
	Rest	Hyperventilation	Rest	Hyperventilation	Rest	Hyperventilation	Rest	Hyperventilation	Rest	Hyperventilation
WH	86.4	87.9	55.6	59.2	11.70	15.60	24	39.0	490	400
JL	80.3	76.6	50.0	52.9	6.95	9.53	18.7	27.6	372	335
TvW	89.4	83.0	45.7	54.2	10.30	14.80	13	33.6	793	440

Voluntary hyperventilation: Voluntary hyperventilation was attempted in 3 patients who hyperventilated during intermittent positive pressure breathing (table 5). Only one study was done in each patient so it is possible that with training a considerably different result might have been obtained. As may be seen in table 5, all of these patients increased their ventilation slightly by increasing their respiratory rate. There was no significant change in carbon dioxide tension in 2 of the patients (*J. L.* and *W. H.*) and a rise in the one (*T. vW.*). In these patients, voluntary hyperventilation was ineffective and, if anything, made the patient worse.

DISCUSSION

The present report is a study of patients with severe emphysema whose disease was in a fairly stable state. This type of patient was chosen because of the difficulty of evaluating any one type of treatment during an acute phase of the illness when all available treatment is utilized at once in order to save the patient. Arterial blood and ventilatory studies were performed before and after intermittent positive pressure breathing on 15 patients, 13 of whom had severe emphysema; 6 had developed a significant degree of carbon dioxide retention. It was found that normal subjects hyperventilate during intermittent positive pressure breathing therapy with oxygen and isopropylarterenol hydrochloride and increase their effective ventilation as shown by a fall in P_{CO_2}. Eight of the patients with severe pulmonary emphysema did not hyperventilate during the therapy and the 5 that increased their ventilation had in most instances less of a response than normal subjects. These findings are similar to those of Motley and associates (15). When hyperventilation occurred in the emphysematous subjects, as in the normal, it was associated with an increase in the tidal volume.

It is interesting that emphysematous patients with a carbon dioxide content below 60 volumes per cent increased their respiratory rate during intermittent positive pressure breathing, while those with severe carbon dioxide retention had a fall in respiratory rate. This difference in response occurred when either oxygen or compressed air was used to activate the apparatus and irrespective of whether hyperventilation occurred. This decrease in respiratory rate may be due to a decrease in the stimulus (oxygen lack) that is thought to control respiration in severely ill emphysematous patients with carbon dioxide retention. However, this leaves unexplained the reason why the respiratory rate returned to the pretreatment level on switching to 50 per cent oxygen at ambient pressure.

In some of the patients with carbon dioxide retention, it was very important whether oxygen or compressed air was used to activate the intermittent positive pressure breathing unit. In those in whom the therapy could not produce hyperventilation, the use of oxygen actually decreased the ventilation and further increased the carbon dioxide tension. One patient (*E. T.*) actually went into apnea a number of times during fifteen minutes of intermittent positive pressure breathing with oxygen. When compressed air was used in these patients, there was no significant change in ventilation or carbon dioxide tension.

Further study of some of those who hyperventilated during intermittent positive pressure breathing therapy showed that the bronchodilator isopropylarterenol hydrochloride was an important contributing factor in producing the increased ventilation. This would suggest that the more patent the airway, especially the finer branches, the greater the likelihood that the tidal volume and thereby the effective ventilation can be increased by intermittent positive pressure breathing. Evidence of improvement of bronchial patency during this therapy with a bronchodilator was also reported by Motley and Tomashefsky (16).

Voluntary hyperventilation in the 3 patients tested failed to reduce the carbon dioxide tension despite the fact that the effective ventilation was increased during intermittent positive pressure breathing therapy in all of these patients. This confirms the study of Wilson and associates (17) who found that normal persons and patients with heart disease could reduce their P_{CO_2} by voluntary hyperventilation, whereas emphysematous patients could not. During voluntary hyperventilation, these patients increased their respiratory rates which, according to Bader and Bader (18), further increases the work of breathing in emphysematous subjects by increasing airway resistance. Riley (19) notes that a small increase in minute ventilation markedly increases oxygen consumption in severe emphysema. He suggests that the increased carbon dioxide tension may be an adaptive mechanism by which the body tolerates an increase in the work of breathing.

The transient nature of the effects produced by intermittent positive pressure breathing is well illustrated in one subject (*J. L.*) who hyperventilated during such therapy. Ventilatory and arterial blood studies obtained within fifteen minutes following cessation of intermittent positive pressure breathing revealed that the values had returned to the pretreatment levels. When given intermittent positive pressure breathing with isopropylarterenol hydrochloride three times daily, using first oxygen and then compressed air for seven and four days, respectively, there was no significant change in the carbon content or tension. Perhaps if continuous therapy had been used, a more prolonged effect might have been attained. However, this might be at the expense of dangerously altered circulatory dynamics even though Cournand and associates (13) found that the maximal circulatory changes occurred within ten to fifteen minutes of initiation of intermittent positive pressure breathing.

Some method of mechanically producing hyperventilation has been recommended repeatedly in severely ill emphysematous patients to reduce the carbon

dioxide tension and at the same time permit needed oxygen therapy. Because of the facility with which it can be used, intermittent positive pressure breathing is usually recommended although the early work was done with the body respirator. There have been many reports (20–22) of the use of a respirator in such a situation. These investigators believed that it was a distinct aid in the management of these cases although the response was usually slow and often equivocal. Noehren (23) reported a case of a patient treated almost continuously with intermittent positive pressure breathing with compressed air along with other measures. Since these patients were treated with all other recommended therapy in addition to mechanical aids to respiration, one wonders how much positive pressure breathing contributed to their recovery.

It has been found that patients whose carbon dioxide is elevated during acute exacerbations of pulmonary insufficiency due to emphysema require a week or so of treatment before the carbon dioxide returns to its previous level. This can occur with a general supportive regimen without oxygen or artificial ventilation. Although these patients are very cyanotic and have oxygen saturations as low as 60 per cent, efforts at improving the airway (intratracheal suction, antimicrobials, bronchodilators, and sometimes tracheotomy) improve the arterial saturation long before the carbon dioxide has changed significantly. The present writers usually administer intranasal oxygen at one liter a minute, as suggested by Barach (10), and continue it if there is no evident depression of the sensorium. Although Barach has stated that he has never observed the development of mental symptoms when delivering oxygen intranasally at this rate, the present writers can recall at least 2 patients who became comatose within fifteen minutes after the institution of such treatment. One of these recovered without the use of oxygen or artificial ventilation. The other was placed in a body respirator and survived. Even in the respirator, intranasal oxygen depressed his sensorium.

Summary

In normal subjects, intermittent positive pressure breathing produces hyperventilation and a reduction in the carbon dioxide tension principally by increasing the tidal volume. Only 5 of 13 patients with severe emphysema hyperventilated during intermittent positive pressure breathing therapy. Again, when this occurred, it was accomplished principally by increasing the tidal volume.

When there is significant carbon dioxide retention due to emphysema, intermittent positive pressure breathing cannot be relied upon to produce the needed hyperventilation. When oxygen was used to activate the apparatus, hypoventilation occurred in 4 of 6 such patients. Whereas the respiratory rate remained unchanged or increased during intermittent positive pressure breathing therapy in patients with no significant carbon dioxide retention, it fell in those who had an elevated carbon dioxide, especially when oxygen was used to activate the unit. This held true whether hyperventilation or hypoventilation occurred in response to intermittent positive pressure breathing therapy.

In the emphysematous patients tested it was found that the concurrent use of isopropylarterenol (Isuprel®) significantly increased the hyperventilatory re-

sponse to intermittent positive pressure breathing. In the one patient with carbon dioxide retention who was studied immediately after intermittent positive pressure breathing was completed, it was found that the blood gases returned to their pretreatment levels within fifteen minutes. Three of the emphysematous patients who responded to intermittent positive pressure breathing with increased ventilation were unable to reduce their carbon dioxide tension by voluntary hyperventilation.

Sumario

Justipreciación de la Capacidad de la Respiración a Presión Positiva Intermitente para Producir Hiperventilación Eficaz en el Enfisema Pulmonar Grave

En los sujetos normales, la respiración a presión positiva intermitente produce hiperventilación y reducción de la tensión del bióxido de carbono principalmente por aumentar el volumen corriente. Sólo 5 de 13 enfermos que tenían enfisema grave hiperventilaron durante la terapéutica a base de respiración con presión positiva intermitente. Además, cuando sucedió esto, se logró principalmente mediante el aumento del volumen corriente.

Cuando existe notable retención de bióxido de carbono debida al enfisema, no cabe confiar en que la respiración a presión positiva intermitente produzca la necesaria hiperventilación. Cuando se usó oxígeno para activar el aparato, hubo hipoventilación en 4 de 6 de esos enfermos. Mientras que la frecuencia respiratoria permaneció inalterada o aumentó durante el uso terapéutico de la respiración a presión positiva intermitente en los enfermos sin mayor retención de bióxido de carbono, bajó en los que había elevación del bióxido de carbono, sobre todo si se usaba oxígeno para activar el aparato. Esto fué un hecho ya sobreviniera hiper- o hipoventilación en respuesta a la terapéutica de la respiración a presión positiva intermitente.

En los enfisematosos comprobados se observó que el uso simultáneo de isopropilarterrenol (Isuprel®) acrecentaba notablemente la respuesta hiperventiladora a la respiración a presión positiva intermitente. En el único enfermo con retención de bióxido de carbono que se estudió inmediatamente después de terminar la respiración con presión positiva intermitente, se observó que los gases sanguíneos volvían a sus valores preterapéuticos en término de quince minutos. Tres de los enfisematosos que respondieron a la terapéutica de respiración a presión positiva intermitente con aumento de la ventilación no pudieron rebajar la tensión de su bióxido de carbono con la hiperventilacion voluntaria.

Resume

Une évaluation de l'aptitude de la respiration sous pression positive intermittente à produire une hyperventilation satisfaisante dans l'emphysème pulmonaire grave

Chez les sujets normaux, la respiration sous pression positive intermittente produit l'hyperventilation et une réduction de la tension du CO^2, surtout par l'augmentation du volume de l'air courant. L'hyperventilation n'a été obtenue que chez 5 malades sur 13 cas d'emphysème sévère durant le traitement par la respiration sous pression positive intermittente. En outre, quand l'hyperventilation s'est produite, c'est l'accroissement du volume de l'air courant qui en a été principalement responsable.

Quand il y a une rétention importante du CO^2 due à l'emphysème, la respiration sous pression positive intermittente ne peut suffire à produire l'hyperventilation nécessaire. Quand l'oxygène a été utilisé pour activer le système, l'hypoventilation s'est produite chez 4 malades dans 6 de ces cas. Alors que la vitesse de la respiration est demeurée la même, ou qu'elle a augmenté durant le traitement par la respiration sous pression positive intermittente chez les malades ne présentant pas de rétention de CO^2 notable, elle s'est abaissée chez ceux où la rétention de CO^2 était élevée, surtout quand l'oxygène était utilisé pour activer le système. Il en était ainsi, soit que l'hyperventilation ou l'hypoventilation

se manifeste comme réaction au traitement par la respiration sous pression positive intermittente.

Chez les malades emphysémateux testés, il a été observé que l'usage simultané d'isopropylartérénol (Isuprel®) augmentait de façon significative la réaction d'hyperventilation à la respiration sous pression positive intermittente. Chez le seul malade présentant un rétention de CO^2 et ayant été étudié immédiatement après l'épreuve de la respiration sous pression positive intermittente, il a été observé que dans les quinze minutes, les gaz du sang reprenaient la valeur qu'ils avaient avant le traitement. Trois des malades emphysémateux, qui répondaient au traitement par la respiration sous pression positive intermittente par une augmentation de la ventilation, étaient incapables de réduire leur tension en CO^2 par l'hyperventilation volontaire.

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DIFFERENTIAL DIAGNOSIS OF BRONCHOGENIC CARCINOMA AND PNEUMONIA IN PATIENTS MORE THAN FORTY YEARS OLD[1]

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INTRODUCTION

Although the final results of surgical resection of bronchogenic carcinoma are discouraging, it is still the best method of treatment. Attempts to improve survival rates by early diagnosis have proved far from satisfactory. One of the most difficult diagnostic problems is differentiating between pneumonia and bronchogenic carcinoma in middle-aged and in elderly patients. It is a common occurrence for patients in this age group with pneumonia to be seen at chest conferences for consideration of thoracotomy because of the possibility of bronchogenic carcinoma. Even at the time of surgery, it is difficult for the surgeon to be certain that a small hidden tumor is not present in an area of infection. Most thoracotomies result in resection of lung tissue. Pulmonary resection in elderly patients may cause more respiratory crippling than resection in younger patients. Thoracotomy should not be undertaken until all other diagnostic procedures have been exhausted. With the hope that some difference in the two diseases might be found, an analysis of the writers' cases has been made and is presented herein.

MATERIAL AND METHOD

The charts of all patients forty years of age or older who were discharged from the hospital as having had pneumonia were reviewed. Patients with bilateral pneumonia were excluded from the study, but patients with unilateral pneumonia of segmental or lobar distribution were included. Some of the patients with pneumonia also had small areas of excavation. No cases of acute pulmonary abscess were considered. The pneumonias were all thought to be bacterial in origin, and *D. pneumoniae*, *H. Streptococcus*, *Staphylococcus aureus*, and *S. viridans* predominated. Sixty-two patients with follow-up observations of two years or longer were found.

The charts of all patients discharged from the hospital with a diagnosis of bronchogenic carcinoma were reviewed. Of approximately 300 patients with such a diagnosis, only 22 patients with roentgenograms of the chest suggesting pneumonia were found. All patients with a diagnosis of bronchogenic carcinoma and a roentgenogram showing primarily a hilar mass or obstruction were deleted from the study. It is a common opinion that bronchogenic carcinoma often resembles pneumonia. If only symptoms are considered, this may be true; however, if the roentgenogram is carefully studied, most of the patients with bronchogenic carcinoma will show a mass or evidence of bronchial obstruction. Only 22, or approximately 7 per cent, of about 300 patients with bronchogenic carcinoma, had roentgenographic findings which truly resembled pneumonia. This means that a selected group of patients with bronchogenic carcinoma were chosen. On several occasions, roentgenograms from the group with pneumonia and from this selected group with bronchogenic carcinoma were shown to chest physicians and radiologists. The correct diagnosis was made in about 50 per cent

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of the cases. Therefore, these patients are thought to be representative of the differential diagnostic problems that occur week after week on a chest service.

The ages of the patients in the pneumonia group varied from forty to seventy-seven years, and averaged fifty-six years. The ages of the patients with bronchogenic carcinoma varied from forty to seventy-five years, and averaged fifty-eight years.

Both groups of cases were analyzed as follows: mode of onset, hemoptysis, weight loss, previous pneumonia, duration of symptoms; physical findings of obstruction, fever, fall of fever to normal, amount of sputum, character of sputum, sputum cultures, hemoglobin, leukocyte count; roentgenographic characteristics; roentgenographic clearing; bronchoscopy; and results of surgery. As cough was present in practically 100 per cent of both groups, no tabulation was made. Dyspnea, at least on exertion, was present also in the vast majority of both groups. An erythrocyte sedimentation test was done on almost all patients at admission, but follow-up sedimentation tests were not done with regularity. In the group of patients with bronchogenic carcinoma, only 12 patients had follow-up erythrocyte sedimentation rates and none returned to normal. Only 10 patients in the pneumonia group had sufficient follow-up erythrocyte sedimentation rates for analysis, and the rates in 3 returned to normal.

The smoking habits of the two groups were analyzed. Five per cent of the 22 patients with bronchogenic carcinoma did not smoke, compared with 12 per cent of the 62 patients with pneumonia. Forty per cent of the patients with bronchogenic carcinoma smoked one-half of a package of cigarettes daily as did 28 per cent of those in the pneumonia group. Twenty-seven per cent of the patients with bronchogenic carcinoma smoked one package of cigarettes daily, compared with 52 per cent of the patients with pneumonia. More than one package of cigarettes was smoked by 27 per cent of the patients with bronchogenic carcinoma and by 9 per cent of the patients with pneumonia.

Results of Analysis

Mode of onset: If symptoms developed suddenly or rapidly within a few days' time the patient was put in the group with rapid onset. Rapid onset of symptoms occurred in 74 per cent of the pneumonia group and in only 26 per cent of the bronchogenic carcinoma group. It was thought that the mode of onset was only suggestive in differentiating the two diseases.

Hemoptysis: Blood-streaked sputum occurred in 35 per cent of the group with pneumonia and in 68 per cent of the group with bronchogenic carcinoma.

Loss of weight: Weight loss (figure 1) was divided into five categories. There is some difference in the percentage showing no weight loss and weight loss of more than 20 pounds. It was noted, however, that patients with pneumonia with symptoms of several months' duration had considerable weight loss. Therefore, the amount of weight loss was probably more closely related to the duration of symptoms than to other factors.

Previous pneumonia: The history of previous pneumonia was similar in both groups. Sixty-three per cent of the pneumonia group gave no history of pneumonia previously and 72 per cent of the bronchogenic carcinoma group had not had pneumonia previously. History of one previous bout of pneumonia was obtained in 24 per cent of the pneumonia group and in 18 per cent of the bronchogenic carcinoma group. Two previous episodes of pneumonia occurred in 10 per cent of the patients with pneumonia and in 5 per cent of the patients with bronchogenic carcinoma. History of three or four previous bouts of pneumonia occurred in only a small percentage of both groups. Two opinions have been

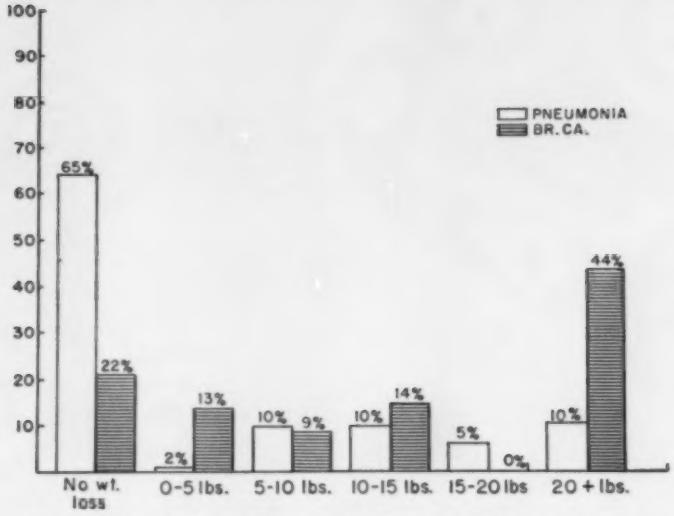

FIG. 1. Chart showing differences in loss of weight in the two groups.

expressed concerning the history of previous pneumonia in bronchogenic carcinoma. One is that patients with bronchogenic carcinoma commonly have a history of repeated pulmonary infection. The other is that a patient more than forty years of age whose first bout of pneumonia clears slowly is more apt to have bronchogenic carcinoma than if his second or third bout of pneumonia clears slowly. No evidence could be found to support either opinion.

Duration of symptoms: There was considerable difference in the two groups (figure 2). This was to be expected. It might be noted that 23 per cent of the patients with bronchogenic carcinoma and 77 per cent of the patients with pneumonia had symptoms for one month or less. Ten per cent of the patients with pneumonia and 50 per cent of the patients with bronchogenic carcinoma had had symptoms for more than four months.

Physical findings: Most reports dealing with bronchogenic carcinoma do not mention physical findings. It is believed that careful physical examination of the chest is of value in differentiating pneumonia from bronchogenic carcinoma. However, careful physical examination of the lungs is in danger of becoming a lost art, and a review of the charts seems to indicate that this is true at this hospital. Available data showed that 13 per cent of the patients with pneumonia had physical findings suggestive of obstruction, and 32 per cent of the patients with bronchogenic carcinoma had findings of obstruction.

Temperature: Fever in the two groups of patients is shown in figure 3. It might be thought that fever would tend to be higher in the group with pneumonia, but there appears to be no significant difference.

Fall of fever to normal is seen in figure 4. Again, there is little difference in

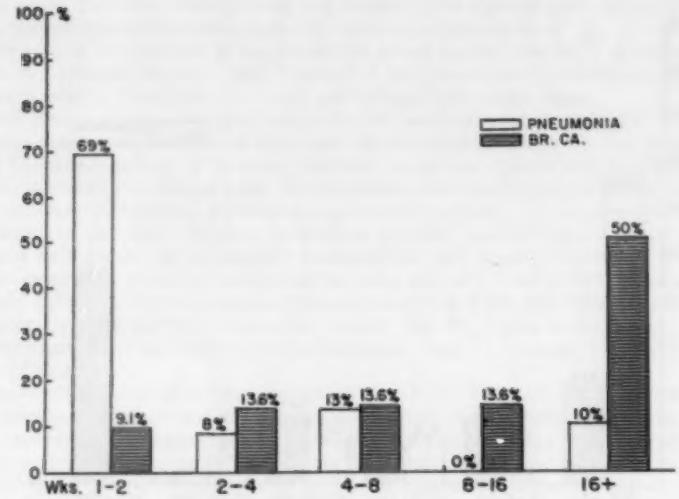

FIG. 2. Chart showing differences in duration of symptoms in the two groups.

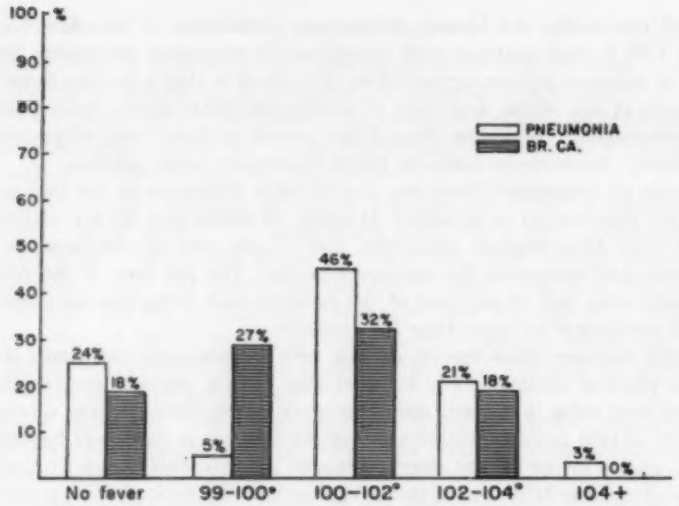

FIG. 3. Chart showing comparison of fever in the two groups.

the two groups except that 93 per cent of the patients with pneumonia were afebrile within three weeks, while only 56 per cent of the patients with bronchogenic carcinoma were afebrile within three weeks. It should be mentioned that most of the patients in both groups were given antimicrobials; however, the

few patients who received no antimicrobials seemed to have had as fast a return to normal temperature as those who received them.

Sputum: The amount of sputum is of some interest (figure 5). Forty-seven per cent of the patients with bronchogenic carcinoma had more than 120 ml. of

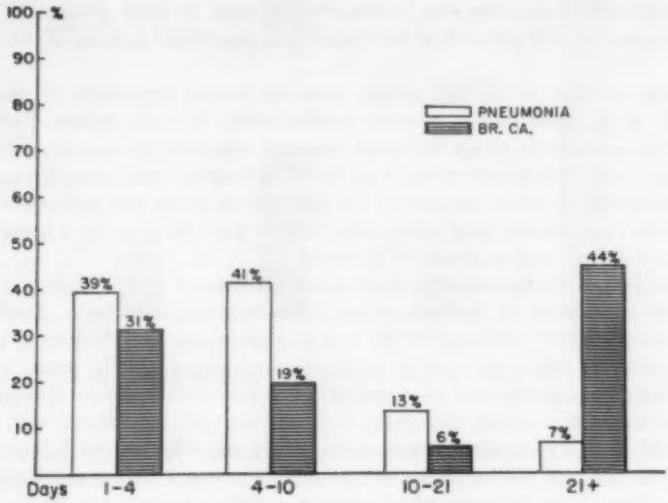

FIG. 4. Chart showing comparison of defervescence in both groups.

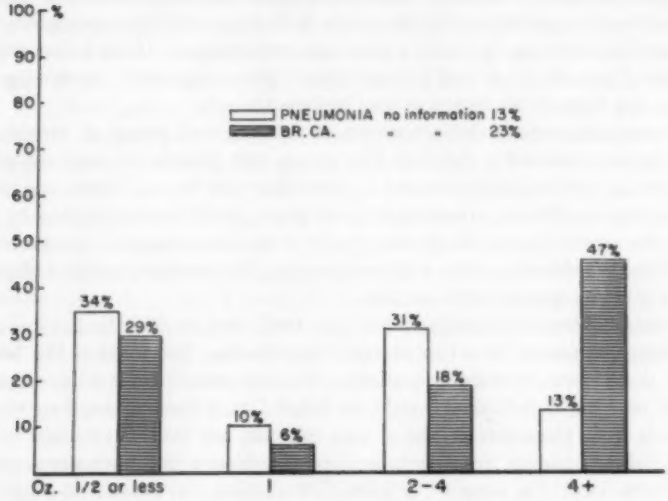

FIG. 5. Chart showing comparison of amount of sputum of patients in both groups.

sputum. Only 13 per cent of the pneumonia group had more than 120 ml. of sputum.

Foul sputum occurred in 18 per cent of the bronchogenic carcinoma group and in 12 per cent of the pneumonia group. Mucoid sputum was noted in 37 per cent of the tumor group and in 28 per cent of the pneumonia group. Purulent and mucopurulent sputum was practically the same in both groups. "Rusty" sputum occurred in 8 per cent of the pneumonia group and in none of the tumor group.

Sputum cultures in the two groups revealed similar organisms: *D. pneumoniae*, *H. streptococcus*, and *S. viridans* predominated in both groups. Forty per cent of the pneumonia group had more than one organism by culture, compared to 33 per cent with mixed cultures in the bronchogenic carcinoma group. The high percentage of mixed cultures in the pneumonia group was unexpected, but it must be remembered that mixed infections appear to occur in a higher percentage of pneumonias in the older patients.

Hematologic data: Hemoglobin determinations showed only a slightly greater tendency to anemia in the group with bronchogenic carcinoma. Leukocyte counts were slightly different in the two groups except that leukocyte counts greater than 15,000 were rare in bronchogenic carcinoma. No patients with bronchogenic carcinoma had an eosinophilia (5 per cent or more). Eosinophilia occurred sometimes during the illness in 33 per cent of the patients with pneumonia. Of the patients with bronchogenic carcinoma who had an initial leukocytosis, 67 per cent had a return of the leukocyte count to normal during the course of the illness. Patients with pneumonia and an initial leukocytosis had a return of the leukocyte count to normal in 94 per cent of the cases.

Roentgenograms: It should be emphasized again that no patient with bronchogenic carcinoma was included in this study if there was definite roentgenographic evidence of obstruction or if only a hilar mass was present. If the lesion appeared to be one of consolidation and if there were slightly suggestive roentgenographic findings of a hilar mass, the case was included.

The roentgenographic characteristics of this selected group of bronchogenic carcinoma are reflected in figure 6. The group with pneumonia was not selected except for age and unilateral lesions. If more than one type of lesion was present, i.e., hilar mass, effusion, consolidation, et cetera, each was tabulated in determining the percentages. Since the group with bronchogenic carcinoma was selected to resemble the group with pneumonia, the roentgenographic characteristics of the two groups were similar.

The term "obstructive pneumonitis" has been used to describe the infiltration in the lung peripheral to a bronchogenic carcinoma. The opinion has been expressed that, since obstructive pneumonitis often causes some contraction of a segment or lobe, this finding would be helpful in differentiating bronchogenic carcinoma from pneumonia. Loss of lung volume, however, is common in pneumonia in older patients. Seventeen per cent of patients with pneumonia revealed some contraction of a segment or lobe. Contraction was present in 23 per cent of the patients with bronchogenic carcinoma and, therefore, was of no help in differentiating the two diseases.

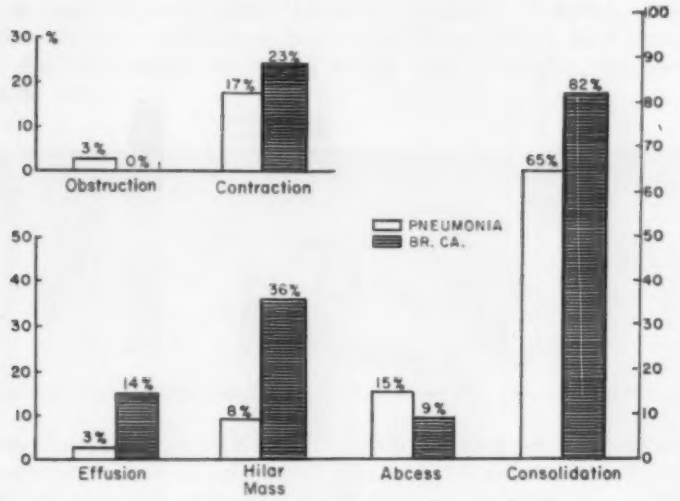

FIG. 6. Chart showing comparison of roentgenographic characteristics of patients in both groups.

Before the days of antimicrobials, pulmonary infection which occurred with bronchogenic carcinoma would not clear, or cleared slowly when compared to the usual rate of clearing seen in pneumonia. After antimicrobials began to be used extensively, it was thought that pulmonary infection occurring with bronchogenic carcinoma would clear quickly and that this valuable differentiating point between pneumonia and bronchogenic carcinoma with pneumonia would be lost. Results shown in figure 7 do not seem to substantiate this opinion. Only 9 per cent of the 22 patients with bronchogenic carcinoma showed more than 50 per cent roentgenographic clearing of the infiltration. One of the patients (*L.W.*, figure 8) had pneumonia involving the right middle and lower lobes. The pneumonia cleared well, but planigrams revealed a 3 by 4 cm. mass at the right hilum.

The second patient (*D.C.*, figure 9), who had less than 50 per cent residual, aspirated some stomach contents during general anesthesia and developed a putrid pneumonia in the right upper lobe and in the superior segment of the right lower lobe. This cleared gradually over a seven-week period. A persistent wheeze was heard on the right and the patient was bronchoscoped, but no tumor was seen. The patient was in poor clinical condition, with chronic bronchitis and pulmonary emphysema, and because of this thoracotomy was not done. It was not until ten months later that a mass was seen at the right hilum.

Ninety-one per cent of the patients with bronchogenic carcinoma showed a residual roentgenographically of more than 50 per cent, and 69 per cent had a residual of more than 75 per cent. Patient *A.P.* (figure 10), is an example of bronchogenic carcinoma that cleared only slightly.

Ninety-six per cent of the 62 patients with pneumonia had a residual roent-

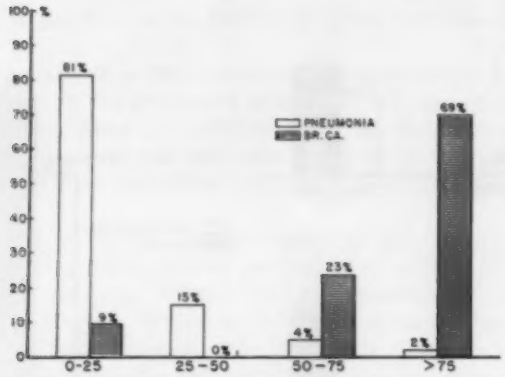

FIG. 7. Chart showing differences in roentgenographic residuals in both groups.

genographically of less than 50 per cent. Patient *S.H.* (figure 11) had more than 50 per cent residual after being followed five weeks. Because of the strong suspicion of bronchogenic carcinoma, a thoracotomy was performed and the left lower lobe was resected. The lesion was inflammatory. The pneumonia in many of the patients cleared slowly, and it should be strongly emphasized that the degree and the rate of clearing are equally important. Patient *C.K.* (figure 12) is an example of pneumonia that cleared slowly but almost completely.

It was thought that pneumonia in patients with chronic bronchitis and emphysema might clear more slowly than pneumonia in patients who did not have this disease. There were 21 patients who had chronic bronchitis and emphysema of variable degree. Eighty per cent of the patients with bronchitis and emphysema had clearing of 50 per cent or more at four weeks after admission to the hospital. Eighty-nine per cent of the patients with no bronchitis and no emphysema had clearing of 50 per cent or more after four weeks' observation. This is a difference of 9 per cent between the two groups of patients, and at six weeks this same 9 per cent difference was found. When the index of 75 per cent or more clearing is used, the difference between the two groups at four weeks' and at six weeks' time is 13 per cent in favor of the patients without bronchitis and emphysema. Hence, it does appear that pneumonia clears more slowly in patients with bronchitis and emphysema.

Bronchoscopic data (figure 13): Only one patient in the bronchogenic carcinoma group had an entirely negative bronchoscopy. Tumor, thickening, fixation, and constriction were common in the presence of bronchogenic carcinoma, but uncommon in the pneumonia group. The only abnormal bronchoscopic findings that occurred with significant frequency in patients with pneumonia were generalized and localized bronchitis. The difference in the bronchoscopic findings in the two groups was unexpected, and it will be of interest to see if approximately these same findings hold in the future.

Surgical results: All except 4 of the patients with bronchogenic carcinoma had

positive tissue diagnoses before thoractomy. Surgery was performed in these 4 patients because of the strong suspicion of tumor. Two of the 4 patients had resectable bronchogenic carcinomas and are still living three and four years following surgery.

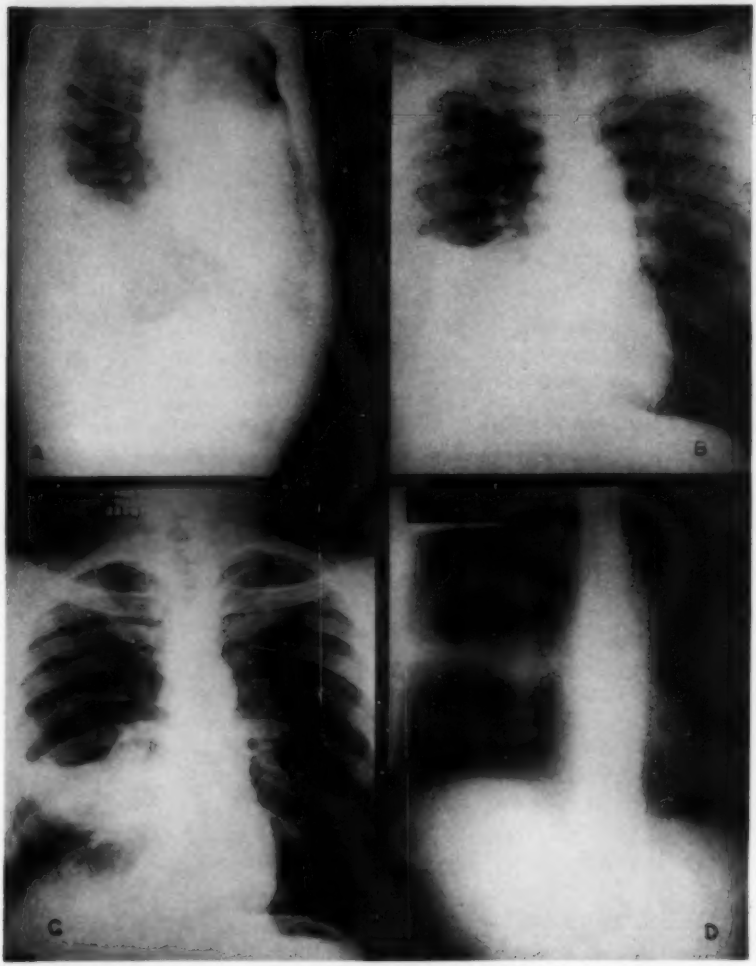

FIG. 8 (L. W.). *A*. Lateral roentgenogram (January 22, 1951), showing infiltration in the right middle lobe and the lower portion of the right lower lobe. *B*. Postero-anterior roentgenogram (January 22, 1951) *C*. Postero-anterior roentgenogram (February 22, 1951), showing partial clearing after one month. *D*. Planigram (February 23, 1951) revealing tumor mass at right hilum.

Patients with symptoms of chronic pulmonary infection severe enough to warrant surgery were not included in this study. Of the 62 patients with pneumonia, 6 were operated upon because of the suspicion of bronchogenic carcinoma. Four lobectomies and two pneumonectomies were performed. One death occurred

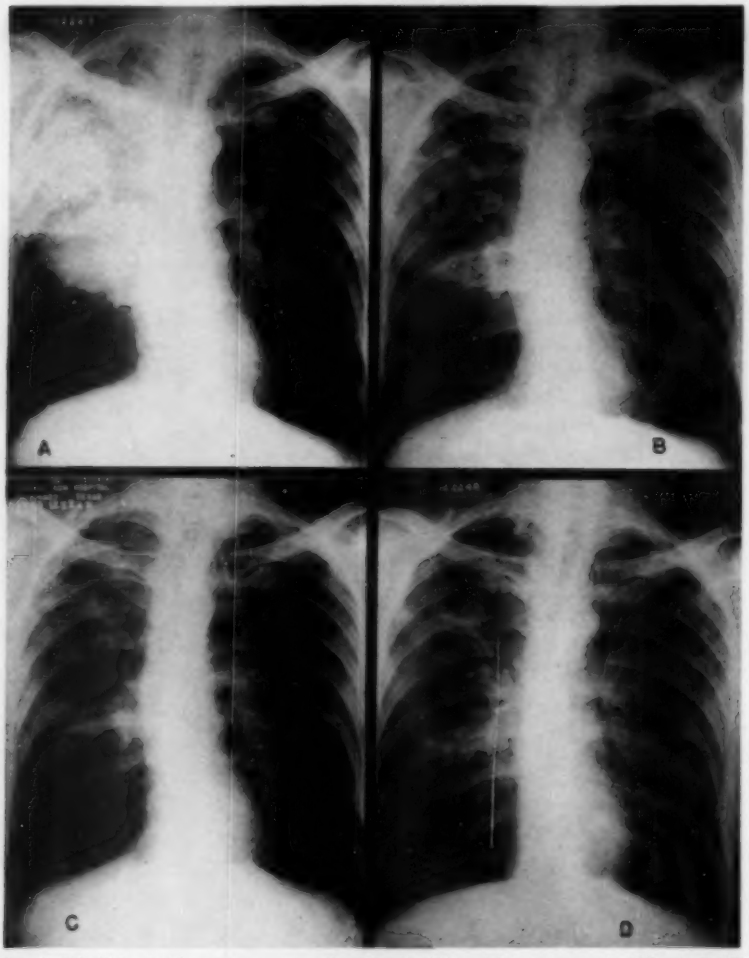

FIG. 9 (D. C.). *A*. Roentgenogram of patient with putrid pneumonia (March 8, 1950), showing right upper lobe and superior segment of right lower lobe. *B*. Roentgenogram (April 28, 1950) showing fair clearing of the pneumonia at seven weeks. *C*. Roentgenogram obtained on November 28, 1950, showing further slight clearing of the pneumonia but some residual in the right upper lobe and at the right hilum. *D*. Roentgenogram (May 26, 1951) showing a spontaneous right pneumothorax and a new mass in the upper portion of the right hilum.

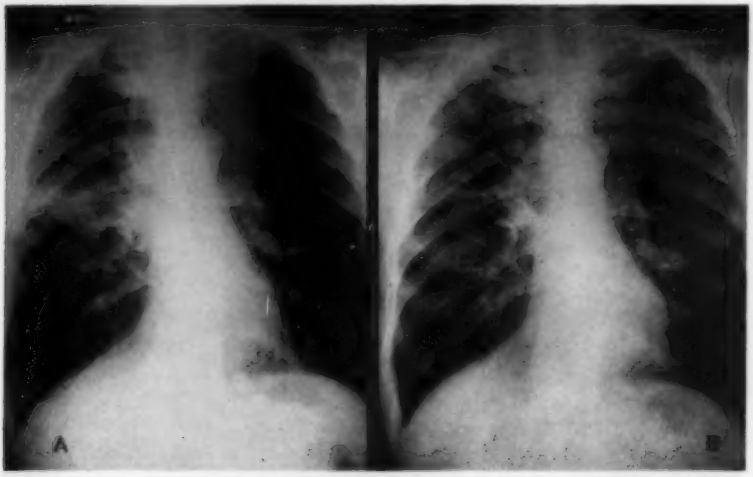

FIG. 10 (A. P.). *A*. Roentgenogram (March 20, 1950) of patient with irregular infiltration in the right middle lung field and at the right hilum. *B*. Roentgenogram (April 28, 1950) obtained five weeks after *A* showing partial clearing in the midlung on the right, but an increase of disease in the areas under the second and third anterior ribs on the right. Infiltration at the right hilum is unchanged.

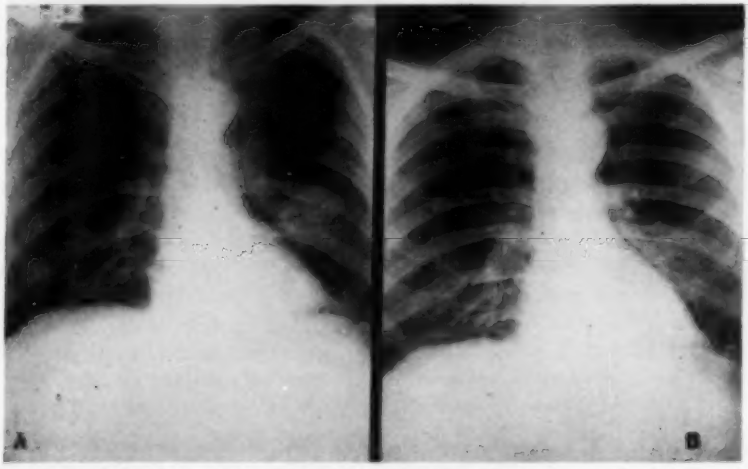

FIG. 11 (S. H.). *A*. Roentgenogram (October 10, 1952) showing round infiltration with irregular border and stranding extending to the left hilum. *B*. Roentgenogram (November 17, 1952) showing decrease in density of the round portion of the infiltration but that the lesion has moved downward toward the left heart border. The area involved remains about the same size.

from what was thought to be serum hepatitis following postoperative transfusion. Two patients had no postoperative complications and were asymptomatic at follow-up examination. Postoperative complications or the extensive resection of pulmonary tissue were permanently disabling in 3 patients.

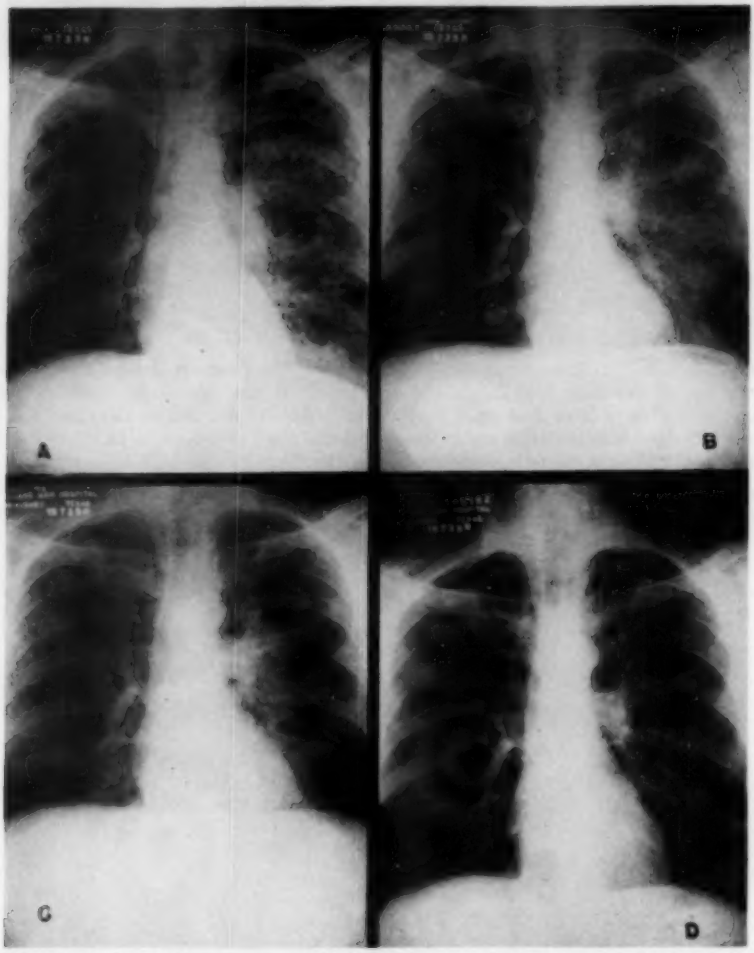

FIG. 12 (C. K.). *A*. Roentgenogram (August 18, 1949), showing increased density at the left hilum and extensive mottling throughout the left lower lobe. *B*. Roentgenogram (September 15, 1949), showing that the mottling has cleared partially but that there is still increased density at the left hilum. *C*. Roentgenogram (November 17, 1949), showing further slight clearing of the mottling along the left cardiac border. The hilar density remains about the same. *D*. Roentgenogram (July 3, 1951), showing that only light stranding remained at a follow-up examination twenty months later.

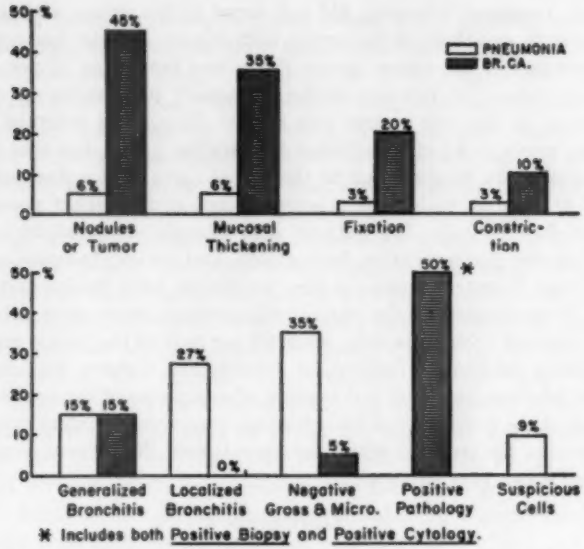

FIG. 13. Chart showing bronchoscopy findings in both groups.

Surgery, therefore, was performed on 10 patients because of the suspicion that bronchogenic carcinoma was present. Four of the 10 patients did have bronchogenic carcinoma and 2 of these are still living three and four years following surgery. One death occurred in a patient with pulmonary infection who had surgery because of the suspicion of bronchogenic carcinoma.

Chronic Localized Pulmonary Suppuration

When this study was started, localized chronic pulmonary suppuration was included in the group of patients with pneumonia. It soon became apparent that chronic suppuration differed in many ways from pneumonia, so that a separate analysis was made. Roentgenograms of most of the patients with chronic suppuration showed some contraction, and occasionally there was a hilar mass if no peripheral adhesions prevented contraction toward the hilum. The greatest difficulty was in differentiating this type of chronic infection from bronchogenic carcinoma that presented as a hilar mass. Therefore, a group of such tumors was analyzed for comparison. Six patients with chronic pulmonary suppuration were found and all of these had surgery.

All patients with chronic suppuration had cough, sputum and a gradual onset of symptoms. Fifteen per cent of the patients with bronchogenic carcinoma that presented as a hilar mass had no respiratory symptoms; 25 per cent had acute onset of symptoms, leaving 60 per cent with gradual onset of symptoms. Duration of symptoms was similar in both groups. Hemoptysis occurred in 66 per cent of the group with chronic suppuration and in 40 per cent of the group

with tumor. Localized wheezing did not occur in the group with suppuration but did occur in one-third of the group with tumor. Weight loss occurred in a higher percentage of the tumor group. Fever was present in 60 per cent of the tumor group and in 100 per cent of the pulmonary suppuration group, but the range of fever in the two groups was similar. Fever had returned to normal within three weeks in all of the chronic suppuration group, but was still present in 60 per cent of the tumor group at the end of three weeks. Sputum tended to be greater in amount with chronic suppuration, and a higher percentage had purulent or foul sputum. No patient with chronic suppuration had mucoid sputum. Cultural characteristics, hemoglobin, and leukocyte count were similar in both groups. Roentgenograms in the two groups were similar and showed no regression. Bronchoscopy in the chronic suppuration group gave either negative findings or showed bronchitis only, while 90 per cent of the tumor group showed tumor, nodular thickening, fixation, or constriction. Surgery was done in all of the chronic infection patients: in 3 because of symptoms of chronic infection and in 3 because of the possibility of bronchogenic carcinoma. Surgery was performed in 35 per cent of the patients with hilar mass tumor; 20 per cent were resectable and one had a five-year survival.

Discussion

The most helpful finding in this study in differentiating bronchogenic carcinoma from pneumonia was the regression of the lesion roentgenographically in pneumonia.

Various criteria are used as indications for thoracotomy in slowly clearing pneumonia. A common belief is that thoracotomy should be done if clearing is not satisfactory in two or three weeks. However, the term "satisfactory clearing" is difficult to define. Of the 62 patients with pneumonia, 26 per cent had a residual roentgenographically of 25 per cent or more at the end of four weeks, and 21 per cent had a residual of 25 per cent or more at the end of six weeks. If 50 per cent or more residual roentgenographically is considered "unsatisfactory clearing," then the percentage of the patients with pneumonia with "unsatisfactory clearing" would be 8 per cent and 6 per cent at four and six weeks, respectively.

Review of the roentgenograms of the patients with pneumonia revealed that, if a decision as to unsatisfactory clearing (50 per cent or more residual) had been made two weeks after admission, 27 per cent would have had "unsatisfactory clearing." If this decision had been made three weeks after admission, 19 per cent would have had "unsatisfactory clearing." Therefore it seems, at least in the group of patients with pneumonia, that, if exploratory thoracotomy is to be held to a reasonable figure, a delay of about four weeks is necessary. Since only 9 per cent of the group of 22 patients with bronchogenic carcinoma resembling pneumonia revealed more than 50 per cent clearing roentgenographically, this also appears to be a fairly accurate indication for thoracotomy in this group.

Planigrams often were helpful in revealing a tumor mass that could not be seen with ordinary roentgenography. In selected patients, angiography would

probably be of value, but it was not used in this study. Gradual onset of symptoms, hemoptysis, and fever of more than three weeks' duration following the start of antimicrobial therapy tended to be more common in bronchogenic carcinoma and might occasionally be helpful in differential diagnosis.

Pneumonia in older patients often clears slowly because chronic bronchitis, emphysema, bronchiectasis, mixed infection, et cetera, are common and interfere with resolution. Therefore, to assume that slowly clearing pneumonia in older patients is due only to bronchogenic carcinoma is unwarranted.

It has been stressed repeatedly that exploratory thoracotomy should be performed whenever there is a suspicion of bronchogenic carcinoma. At this hospital there has been only one instance of a patient with pneumonia in whom a so-called exploratory thoracotomy was done and nothing resected. With all other patients the surgeons were not able to rule out definitely a small hidden tumor, and the lesion was resected. The present writers believe, therefore, that every effort should be made to select patients carefully for thoracotomy. However, there will be patients with pneumonia in whom the suspicion of bronchogenic carcinoma is so strong that waiting to see if regression of the lesion takes place is not justified. Even if satisfactory clearing does take place in older patients with pneumonia, frequent roentgenograms must be taken to prevent misdiagnosis of unusual cases of bronchogenic carcinoma with infection.

It should be stressed that, if a decision is made to delay thoracotomy for a few weeks to see if roentgenographic clearing will occur, the patient must remain in the hospital. Patients should be bronchoscoped, secretions taken for observation of tumor cells, and frequent roentgenograms should be made. Planigrams should be obtained to determine whether a tumor mass or bronchial obstruction is present. However, bronchial obstruction frequently can be better demonstrated by localized bronchograms made with a soluble opaque solution and the use of spot roentgenograms to give better detail. The patient should be discharged from the hospital only after all of the evidence is in favor of a slowly clearing pneumonia and against a bronchogenic carcinoma. Even after discharge from the hospital, patients must be recalled for frequent follow-up examinations.

Chronic pulmonary suppuration simulates bronchogenic carcinoma so closely that it is difficult or impossible to differentiate between the two, and thoracotomy is usually indicated.

Summary

Slowly clearing pneumonia in older patients is common and is due to many causes. Pneumonia in patients more than forty years of age usually shows roentgenographic clearing of more than 50 per cent at four or six weeks. Bronchogenic carcinoma resembling pneumonia usually shows roentgenographic clearing of less than 50 per cent at four to six weeks.

Evaluation of the patients with pneumonia in the present study two and three weeks after admission revealed about 25 to 20 per cent with unsatisfactory roentgenographic clearing.

Gradual onset of symptoms, hemoptysis, slow defervescence, and physical

findings of obstruction are suggestive only in differentiating pneumonia and bronchogenic carcinoma. Even when a positive histologic diagnosis could not be made following bronchoscopy, the presence of thickening, fixation, and constriction appeared to be strongly suggestive of bronchogenic carcinoma.

It is practically impossible to differentiate localized chronic pulmonary suppuration from bronchogenic carcinoma. Consequently, a most careful selection of patients for thoracotomy should always be attempted.

Sumario

Diagnóstico Diferencial del Carcinoma Broncógeno y de la Neumonía en Sujetos de Más de Cuarenta Años de Edad

La neumonía de desaparición lenta es frecuente en las personas de mayor edad, reconociendo muchas causas. En los enfermos de más de cuarenta años de edad, la neumonía suele mostrar despejo radiográfico de más de 50 por ciento a las cuatro a seis semanas. El carcinoma broncógeno parecido a la neumonía suele mostrar despejo radiográfico de menos de 50 por ciento a las cuatro a seis semanas.

En el estudio actual, una justipreciación de los enfermos neumónicos a las dos y tres semanas de su ingreso reveló aproximadamente 25 a 20 por ciento con despejo poco satisfactorio en la radiografía.

La iniciación gradual de los síntomas, la hemoptisis, la baja lenta de la fiebre a lo normal y los hallazgos físicos de oclusión no son más que indicativos en la diferenciación de la neumonía y el carcinoma broncógeno. Aun cuando no cabía hacer un diagnóstico histológico positivo en la broncoscopia, la existencia de espesamiento, fijación y contracción parecían sugerir elocuentemente carcinoma broncógeno. Es casi imposible diferenciar de éste la supuración pulmonar crónica localizada. Debe tratarse siempre de hacer una selección cuidadosa de los enfermos destinados a la toracotomía.

Resume

Diagnostic différentiel du cancer bronchogène et de la pneumonie chez les malades âgés de plus de quarante ans

Chez les malades âgés, l'infiltrat pneumonique s'éclaircit en général lentement et cela est dû à de nombreuses causes. Chez les malades ayant plus de quarante ans, les clichés radiologiques montrent habituellement la disparition de 50% des opacités dans les quatre à six semaines. Dans le cancer bronchogène ressemblant à la pneumonie, la disparition des opacités n'atteint pas 50% en quatre ou six semaines.

Dans l'étude actuelle, l'évaluation des cas de pneumonie, deux et trois semaines après l'entrée à l'hôpital, révèlait qu'il y avait environ 25 à 20% des cas où l'éclaircissement des radiogrammes n'était pas satisfaisant.

Le début progressif des symptômes, l'hémoptysie, le retour lent de la température à la normale et la découverte d'une obstruction d'ordre physique ne sont significatifs que lorsqu'il s'agit de différencier la pneumonie du cancer bronchogène. Même dans le cas où le diagnostic par une biopsie positive ne pouvait être obtenu en bronchoscopie, la présence d'un épaississement, d'une rigidité ou d'une constriction paraissait fortement en faveur du diagnostic de cancer bronchogène. Il est pratiquement impossible de différencier la lésion supurative chronique du poumon du cancer bronchogène. Une sélection prudente des malades devant être soumis à la thoracotomie est toujours nécessaire.

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MINIMAL PULMONARY TUBERCULOSIS

An Early Evaluation in the Recent Antimicrobial Era

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Introduction

Early diagnosis and successful treatment of active minimal pulmonary tuberculosis are perhaps the most important factors in tuberculosis control other than prophylaxis. Whether successful treatment includes the use of surgery in all cases of minimal tuberculosis is still an unresolved issue. The pathologic and bacteriologic characteristics of the resected specimens have prompted the assumption that most, if not all, of these residual foci should be resected for the maximal benefit of the patient. Reports thus far available fail to show any convincing difference in relapse rate whether these patients underwent surgical treatment or not. The lack of appreciable difference in relapse rate in the surgical and nonsurgical patients, plus a mortality rate of 1 per cent or more in segmental resections and a significant morbidity in those undergoing surgical treatment (1), has prompted many studies to determine which patients are actually in need of surgical treatment. Reliable data may have to await the results of a study of comparable series using randomization technique, as started by Raleigh and reported at the Twelfth, Thirteenth, and Fourteenth Veterans Administration Tuberculosis Chemotherapy Conferences. Unfortunately, in view of the prolonged follow-up period advisable in such a group, these results will not be available for years.

The present paper was stimulated by both impatience and curiosity. It is a retrospective study of all adult patients, 95 in number, who had minimal pulmonary tuberculosis (2) and were admitted and treated at Nopeming Sanatorium, Nopeming, Minnesota, during the four-year period 1951 to 1954, inclusive. Excellent studies were done on this phase of the disease before chemotherapy was available (3–6).

Minimal cases were chosen for study because the initial disease and residual focus seemed to contain fewer variables than in those patients who had more advanced disease. Furthermore, if resection was performed, it seemed likely that little disease would remain in the lung as a basis for future relapse. It is also in the minimal group that the medical and surgical approaches are likely to be most divergent. The study has all of the limitations of a retrospective review of nonrandomized cases.

Clinical Material

Fourteen of the 95 patients had been treated previously either elsewhere or at Nopeming but without chemotherapy. There have been no deaths. All pa-

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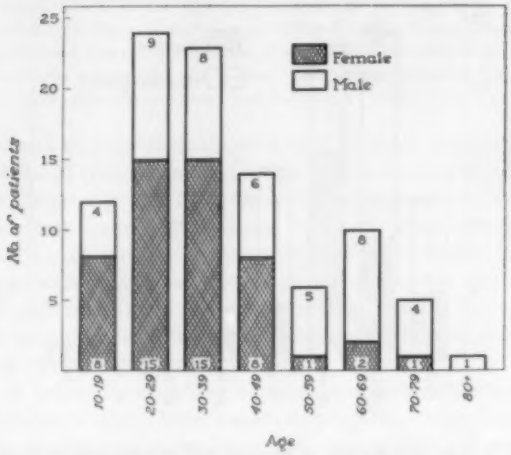

FIG. 1. Age and sex distribution of 95 patients who had minimal pulmonary tuberculosis.

tients had routine stereoroentgenograms and appropriate lateral films on admission, as well as planigrams as indicated.

No attempt has been made to evaluate the relative efficacy of various chemotherapeutic combinations. The 95 patients were treated by two or more agents in addition to a modified regimen of rest in bed.

Duration of treatment was as follows: less than six months, 9 patients (all in 1951); six to twelve months, 43; twelve to eighteen months, 35; more than eighteen months, 8 patients.

The relative frequency of various combinations of medications employed was as follows: dihydrostreptomycin and para-aminosalicylic acid (PAS), 53 patients; isoniazid-PAS, 5 patients; all three of the foregoing, 28 patients; and isoniazid-dihydrostreptomycin, 9 patients.

Thirty-five patients with minimal disease underwent resection of lung tissue as part of their treatment. The indications by which these patients were selected for resection by the medical staff of the sanatorium were never rigid. Factors which influenced the decision from time to time were the slowness of attaining a noninfectious status and the type or personality of the patient, as well as the decrease in enthusiasm for surgical treatment in this type of case which gradually arose both at the sanatorium and throughout the country. The difficulty in predicting the ultimate resolution under chemotherapy became increasingly apparent.

The great majority of operations were segmental resections. Lobectomy was the most extensive procedure performed. Temporary or permanent collapse procedures were not done.

Population: There were 50 females and 45 males in the group. Age distribution by sex and by surgical or nonsurgical treatment is shown in figures 1 and 2.

Eighty-eight patients were white and 7 were non-white. Of the 95 there were

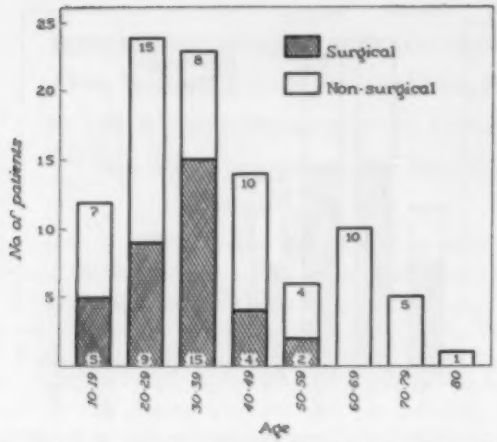

FIG. 2. Age distribution of surgical and nonsurgical patients.

eight nurses and one physician. The remainder were mostly laborers and housewives.

Case finding: The high percentage of cases disclosed by survey roentgenographic methods was encouraging and, it is believed, was significant. It suggests again that survey methods are the best means of discovering patients in the minimal stage of their disease. The survey roentgenograms included films obtained by mobile unit and hospital admission photofluorograms, as well as those required of all persons admitted to the county jail, work farm, and county home. In addition, many local industries require pre-employment and annual roentgenograms.

Cases discovered by survey methods may be broken down as follows: survey-type films, twenty-seven; jail commitment, seven; hospital admission, three; pre-employment and employment, nine; making a total of forty-six.

OBSERVATIONS

Bacteriologic diagnosis on admission: Only bacteriologic data on admission to the hospital will be presented. The final diagnostic criterion was production of tuberculous lesions in the guinea pig. Eleven of the 95 patients failed to have positive bacteriologic diagnoses established. These 11 patients all had positive Mantoux tests, roentgenographic changes compatible with tuberculosis, typical roentgenographic clearing under chemotherapy, and in most cases definite history of contact with tuberculosis. Four of the 11 patients underwent segmental resection, and in each case the diagnosis was confirmed from the specimen bacteriologically.

Sputum was concentrated by the sodium hydroxide method. Culture specimens were inoculated into three tubes of Lōwenstein medium and one of Petragnani medium. If

microscopic examination of the sputum concentrate was negative, three gastric aspirations were performed. Two of these were cultured and one was inoculated into a guinea pig. When the patient was raising sputum, three sputum cultures were done. If both sputum and gastric cultures were done, the patient had twenty cultures running, including four tubes each of three sputum cultures and four tubes each of two gastric cultures.

In 57 patients all four methods were used to study the pulmonary secretions. In only 3 of the 57 patients were tubercle bacilli demonstrated by all four methods of study. In no case were tubercle bacilli subsequently isolated from a patient who had not been discharging tubercle bacilli when admitted to the hospital.

Reversal of infectiousness ("sputum conversion"): Eighty-four patients were discharging tubercle bacilli on admission to the hospital. Seventy (83 per cent) became noninfectious within one month after admission. By five months 94 per cent and by seven months 99 per cent had attained noninfectious status. These "sputum conversion" rates are illustrated in figure 3.

Location of lesion: Localization of roentgenographically demonstrable disease was as follows: right upper lobe, 34; left upper lobe, 36; bilateral upper lobes, 22; other, 3. The localization of the lesion had no apparent influence on the prognosis or stay in the hospital.

Duration of stay in the sanatorium: Eighty-seven patients were dismissed with the approval of the medical staff. The wide range in stay in the hospital of these patients illustrates how the treatment in each case was individualized. Stability of both the patient and the lesion was a major factor in considering the time of dismissal.

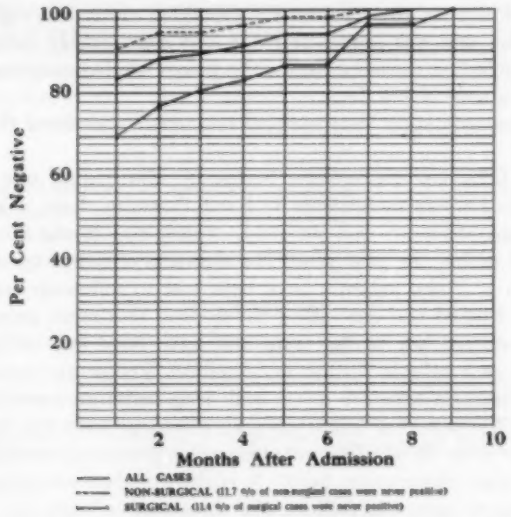

FIG. 3. Percentage of 84 patients with demonstrable tubercle bacilli on admission who had become noninfectious by the designated periods after admission.

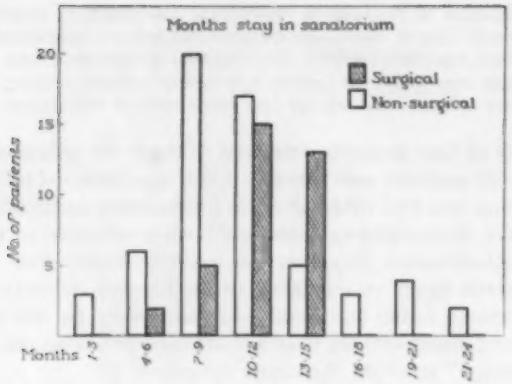

FIG. 4. Distribution of patients by duration of stay in the sanatorium.

Eight patients left the sanatorium against medical advice. In one of these patients the disease subsequently relapsed. These 8 patients have not been lost to follow-up observation.

The average hospital stay in days was as follows: surgical patients, 356.2; nonsurgical patients, 311.9; all patients, 328.2.

The extremes in duration of stay in the sanatorium (figure 4) lie in the nonsurgical group. Of the 3 held less than three months, one left the sanatorium against medical advice, and 2 (one of whom was an elderly physician) were dismissed to home care. Of the 6 patients hospitalized more than eighteen months, 4 were alcoholic, one was senile, and one was considerably below average in intelligence. Excluding these extremes, the group treated nonsurgically had an average hospital stay of 298 days.

There was no indication that surgical treatment shortened the stay in the sanatorium.

Most recent follow-up observations: Follow-up observation was accomplished by the institution's own facilities in all but 6 cases; in these, a letter from the patient's private physician was obtained. Ninety-two of the 95 patients have been examined within the past year. The duration of follow-up observations is shown in table 1. Three patients have been lost to follow-up; of these, 2 had been surgically treated and one was in the medical treatment group. Two of the patients have moved out of the state and have been lost to follow-up. One patient in the area refuses further examination. Follow-up periods are necessarily limited because effective drugs and drug regimens have been available only recently. The period of follow-up since discharge from the sanatorium was very similar for both the surgical and nonsurgical groups up to and including the two- to three-year observation point. A slightly higher percentage of medical cases than surgical cases was followed for three to five years.

Relapses: Seven instances of relapse of the tuberculosis were encountered. The criteria for relapse are as follows: reappearance of tubercle bacilli in sputum

TABLE 1

INTERVAL FROM DISMISSAL FROM THE SANATORIUM TO THE LAST FOLLOW-UP OBSERVATIONS

Interval	Patients
years	
Less than 1	26
1–2	24
2–3	28
3–4	12
4–5	2
Lost	3
Total	95

TABLE 2A

SURGICAL RELAPSES

Case	Chemotherapy Before Surgery	Type of Relapse	Months After Operation	Comment
4	30 gm. of dihydrostreptomycin with PAS in 1 month	6 sputum concentrates, positive for tubercle bacilli over 3-month period	4	Repeat course of dihydrostreptomycin with PAS without further relapse
32	40 gm. of dihydrostreptomycin with PAS in 2 months	1 gastric culture positive for tubercle bacilli, week of delivery	36	Re-treated with isoniazid and PAS
49	50 gm. of dihydrostreptomycin with PAS in 3 months	1 sputum concentrate and 1 gastric guinea pig positive for tubercle bacilli	6	Dihydrostreptomycin and PAS continued without further relapse
65	35 gm. of dihydrostreptomycin with PAS in 4 months	2 sputum concentrates reported Gaffky III, 2 weeks apart	½	Dihydrostreptomycin and PAS continued without further relapse

TABLE 2B

TIME OF RELAPSE SHOWN IN NUMBER OF MONTHS FOLLOWING ADMISSION, SURGERY, AND DISCHARGE

Relapse in Surgical Cases				Relapse in Nonsurgical Cases		
Case Number	Months Post Admission	Months Post Surgery	Months Post Discharge	Case Number	Months Post Admission	Months Post Discharge
4	7	4	—	2	14	3
32	41	36	30	35	15	5
49	9	6	—	44	33	24
65	3½	½	—			

TABLE 3

NONSURGICAL RELAPSES

Case	Hospital Days	Chemotherapy Before Relapse	Type of Relapse	Months After Discharge	Comment
2	298	32 gm. of dihydrostreptomycin with PAS in 1 month	Roentgenographic spread only	3	Retreated with rapid clearing of roentgenographic lesion
35	337	70 gm. of dihydrostreptomycin with PAS in 8 months	1 sputum culture positive for tubercle bacilli	5	Retreated with rapid reversion to negative
44	278	97 gm. of dihydrostreptomycin with PAS in 9 months	1 sputum and 1 gastric culture positive for tubercle bacilli	24	Retreated with rapid reversion to negative. Alcoholic, left hospital without leave

or gastric specimens; roentgenographic evidence of new or progressive disease; the development of extrapulmonary tuberculous disease. There were no cases in this third category.

There were four surgical relapses, which are detailed in table 2*A*. *Case 65* (*see* table 2*A*) is classified as a relapse case by definition, although the relapse is very likely not significant. Bacteriologic examination of resected specimens in the foregoing cases was not consistent or prognostic. The surgical specimen in *Case 49* was negative for tubercle bacilli by smear, culture, guinea pig inoculation, and acid-fast control stains, but other lesions were palpated which were not resected. In *Cases 4* and *65* the tissue was positive for tubercle bacilli by culture alone and in *Case 32* by tissue smear alone.

The optimal time for operation has not been determined. It is believed to be significant that 3 of the 4 patients whose disease relapsed underwent operation within four months of admission to the sanatorium.

All relapses responded rapidly to continued or renewed chemotherapy. In 3 of the 4 surgically treated patients whose disease relapsed, the relapse occurred while the patient was still in the sanatorium. (*See* details in table 2 *B*.)

Relapses in the nonsurgically treated patients are detailed in table 3. These patients were treated early in the series with drug regimens which would now be regarded as "inadequate"[3] both in duration and total amount. The relapses responded rapidly to further treatment. The patient in *Case 44* was an alcoholic

[3] A statement by the Committee on Therapy of the American Trudeau Society (Am. Rev. Tubere., 1955, *72*, 408): ". . . continuous antimicrobial therapy should be maintained for long periods of at least twelve months and most often for eighteen months or even longer."

who left the hospital on his own decision and was lost to follow-up for a two-year period. His disease may have relapsed much earlier than noted, but evidence on the point is not available. The patient was finally returned for further treatment through our jail-survey program. Note that relapse of the tuberculosis occurred in only one of the 4 surgically treated patients after dismissal from the sanatorium. The nonresection group had no relapses in the sanatorium, and in no patient who was given adequate chemotherapy by present standards has the tuberculosis relapsed to date.

TABLE 4

DETAILS OF SURGICAL COMPLICATIONS

Surgical Number	Months of Chemotherapy Preoperatively	Complications	Remarks
2	8	Postoperative hemothorax	*a*. Postoperative transfusion, 1,000 ml. of blood *b*. Enzymatic débridement using streptokinase and streptodornase
4	11	*a*. Complete atelectasis, operated lung *b*. Empyema postoperatively	*a*. Bronchoscopy *b*. Decortication 1 month postoperatively
6	3	Wound infection	Closed after 6 weeks' drainage
13	3	Persistent apical pneumothorax pocket	Disappeared after 5 months
14	4	*a*. Hemopneumothorax *b*. Decortication within 1 month of resection—multiple bronchopleural fistulas found *c*. Apical pneumothorax pocket after decortication	*a*. Decortication *b*. Pneumoperitoneum used in effort to obliterate persistent pneumothorax pocket
18	2	*a*. Postoperative atelectasis in operated lung *b*. 50 per cent collapse by pneumothorax postoperatively	*a*. Bronchoscopy on eighth and tenth days postoperatively *b*. Reinsertion of intercostal catheter 1 week postoperatively for 6 days
21	7	Postoperative hemothorax with incomplete expansion of lung	Decortication attempted but 4-rib thoracoplasty done 2 months postoperatively
26	7	Complete collapse by pneumothorax 1 week postoperatively	*a*. "S" needle with catheter and Stedman suction used three times *b*. Pneumothorax persisted about 1 month

Bacteriology of the resected specimens: Thirty-eight specimens were obtained from 35 patients. In 2 cases the specimens were not studied bacteriologically.

Four methods of study were used: smear, culture, guinea pig inoculation of macerated specimens, and acid-fast control stains of tissue sections. Justifiably or not, acid-fast debris and atypical beaded acid-fast bacilli were called "positive." Cultures were inoculated on three tubes of Löwenstein medium and one tube of Petragnani medium. One guinea pig was used per specimen.

Thirty-two of the 36 specimens studied (89 per cent) were positive for tubercle bacilli by one or more methods. Viable acid-fast bacilli were demonstrated in 9 (26 per cent) of the 35 specimens studied by either culture or guinea pig inoculation.

Viable bacilli were more frequently seen in specimens from patients who had had chemotherapy for four months or less. In this group 5 of the resected specimens were positive for *M. tuberculosis* on culture and 6 were negative. Three of the resected specimens from those who had had chemotherapy for more than four months contained culturable tubercle bacilli, whereas the cultures of 21 of the specimens were negative. Three of the 38 specimens showed evidence of a small cavity which had not been diagnosed preoperatively.

Surgical complications: Eight of the 35 surgical patients (23 per cent) suffered significant complications following operation. Six of the 8 required further operative procedures. There were no deaths in this small series. The mortality rate appears to vary between 0.5 and 1 per cent in the experience of others doing subsegmental and segmental resections. The details of the surgical complications are given in table 4.

Discussion

In the present series of 95 minimal tuberculous pulmonary infections there were two peaks of population, one in the twenty-to-forty-year group and the other in the sixty-year or older group. Most of the young patients were female and most of the older patients were male. There was a high incidence of professional medical personnel (nine).

Survey-type films accounted for about one-half of the 95 cases reported. Case and contact follow-up observations and private physician referral each acounted for about one-fourth of the series.

When each specimen was inoculated into four tubes of standard culture medium, sputum and gastric cultures produced the highest percentage of positive bacteriologic findings; in 88 per cent of the cases, tubercle bacilli were demonstrated.

Roentgenographically demonstrable lesions occurred with approximately equal frequency in either of the upper lobes; bilateral upper-lobe disease was present in 23 per cent of the group. Only 3 patients had lesions in other than the upper lobes.

Of the bacteriologically proved cases, 83 per cent of the patients became noninfectious within one month of admission; 94 per cent were noninfectious within five months; and all but one patient had attained a noninfectious status

within seven months. In the Veterans Administration series, a maximum of 61 per cent of the patients have negative bacteriologic findings at four months and 89 per cent have negative findings at five to eight months on the various two-drug regimens (7).

The average stay in the hospital of the surgical group was longer than the stay of those not resected (356.2 days versus 311.9 days).

The over-all relapse rate was 7.4 per cent. Four of the surgical cases showed evidence of relapse (11.4 per cent), and 3 of the nonsurgical cases showed evidence of relapse (5.0 per cent). The duration of chemotherapy was inadequate by current standards in at least 6 of the 7 patients whose disease relapsed. In the 4 surgical cases in which relapse occurred the treatment had consisted of one, three and one-half, nine, and eleven months of chemotherapy. The three medically treated patients with relapse had had one, eight, and nine months of chemotherapy. Three of the four relapses in surgically treated patients were characterized by the reappearance of tubercle bacilli within six months after operation, and one relapse represented a single gastric culture of tubercle bacilli obtained during the week of delivery two and one-fourth years after resection and adequate chemotherapy. In all 7 patients, the discharge of tubercle bacilli was only transient, and all of the lesions responded rapidly to continued or renewed chemotherapy.

There was no instance of progression of disease during treatment. No evidence was found that surgical treatment improved the outlook or decreased the relapse rate in these patients. Furthermore, 23 per cent of those who underwent operation had significant complications, although there were no deaths. It might be suspected that the surgical group includes those with maximal disease in the minimal classification. There was no evidence to support such a viewpoint. Virtually none of the patients in either group showed complete roentgenographic clearing. No further attempt at roentgenographic classification was made because of the known unreliability of the method.

The data presented suggest that the surgical group may have been positive for tubercle bacilli slightly longer than the nonsurgical group. On the other hand, the nonsurgical group included a number of cases of a type that has always been difficult to treat. These are represented by severe alcoholics, behavior problems, and the older age group, as well as 5 patients who refused surgical treatment. The disease of the 5 patients, incidentally, has not relapsed. Relapse occurred in one of 8 patients with an unsanctioned discharge from the hospital.

In view of the lack of difference of relapse rate at this observation period in the two groups, plus a 23 per cent morbidity rate and a 1 per cent mortality rate in the surgical groups, the present writers question whether surgical resection of minimal pulmonary tuberculosis is justifiable. If the procedure is justifiable, more clear-cut criteria for the selection of the minimal case requiring operation are certainly desirable.

Viable organisms were demonstrated by standard culture and animal inoculation techniques in 26 per cent of resected specimens, and 89 per cent showed evidence of acid-fast organisms. Patients who had undergone chemotherapy of

four months' or less duration had surgical specimens with viable organisms (5 of 11) more often than did those who had treatment for longer than four months (3 of 24).

Three of the four relapses in surgically treated patients occurred in patients who had undergone operation within four months of admission to the sanatorium.

Summary

The cases of 95 adult patients who had minimal pulmonary tuberculosis that was treated with chemotherapy between 1951 and 1954 have been reviewed. Roentgenographically demonstrable lesions were seen equally in either upper lobe and, in one-fifth of the cases, the lesions were bilateral. Almost one-half of the cases of minimal disease were found by means of survey films. Of all admission bacteriologic studies, sputums and gastric cultures were the most frequent source of tubercle bacilli. Reversal of infectiousness was achieved in most cases within one month after admission. Relapses were benign and readily controlled. There were no deaths or progression of disease. In the surgically treated group more viable tubercle bacilli were recovered from the resected specimen, and more relapses were seen if the preoperative treatment had been less than four months than if it had been more than four months. The surgical patients did no better than the nonsurgical patients; indeed, 23 per cent had significant surgical complications. From this experience the writers have been unable to define the indications for resection of minimal pulmonary tuberculosis.

Sumario

Tuberculosis Pulmonar Mínima: Una Justipreciación Temprana en la Reciente Era Antimicrobiana

Pásase revista a 95 enfermos adultos que tenían tuberculosis pulmonar mínima tratadá con quimioterapia entre 1951 y 1954. Discútense las características de la población. Notáronse por igual lesiones descubribles radiográficamente en uno u otro lóbulo superior, y en la quinta parte de los casos las lesiones eran bilaterales. Casi la mitad de los casos de enfermedad mínima fueron descubiertos con radiografías de encuesta. Los cultivos del esputo y gástricos resultaron ser más a menudo los positivos en los estudios bacteriológicos de ingreso. En la mayoría de los casos, se obtuvo viraje de la infecciosidad en término de un mes del ingreso. Las recidivas fueron benignas y fáciles de dominar. No hubo mortalidad o agravación de la dolencia. En el grupo tratado quirúrgicamente, se obtuvieron más bacilos tuberculosos viables del ejemplar resecado y se observaron más recidivas cuando el tratamiento preoperatorio había durado menos de cuatro meses que cuando había durado más de cuatro. Los enfermos quirúrgicos no lo pasaron mejor que los otros; es más, 23 por ciento tuvieron importantes complicaciones quirúrgicas. Los AA. no han podido definir las indicaciones para la resección en la tuberculosis pulmonar mínima.

Resume

Tuberculose pulmonaire a minima; Evaluation précoce au cours de l'ère antibactérienne récente

Analyse de 95 cas de tuberculose *a minima* de l'adulte traités par la chimiothérapie entre 1951 et 1954. Les caractéristiques de la population sont étudiés. Les lésions visibles aux rayons X étaient également réparties dans l'un ou l'autre lobe supérieur, dans un

cinquième des cas elles étaient bilatérales. Presque la moitié des cas furent découverts par le dépistage radiologique systématique. Parmi les examens bactériologiques effectués à l'entrée à l'hôpital, ce sont les cultures de crachat et de lavage gastrique qui étaient le plus souvent positifs. La négativation de l'expectoration a été obtenue dans la plupart des cas dans le mois qui a suivi l'hospitalisation. Les rechutes furent bénignes et facilement contrôlées. Il ne s'est produit aucun décès ou progression de la tuberculose. Dans le groupe traité chirurgicalement, le nombre des bacilles viables récupérés dans les pièces réséquées était plus grand, et les rechutes étaient plus fréquentes, quand la durée du traitement préopératoire était inférieure à quatre mois que lorsqu'elle dépassait quatre mois. L'état des malades opérés n'était pas meilleur que celui des malades non opérés; en fait, 23% des cas opérés ont présenté des complications chirurgicales significatives. Les auteurs ne sont pas parvenus à définir les indications de la chirurgie d'exérèse dans la tuberculose pulmonaire *a minima*.

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HOMOLOGOUS AND HETEROLOGOUS IMMUNITY IN INFECTIONS OF MICE WITH MYCOBACTERIUM ULCERANS AND MYCOBACTERIUM BALNEI[1]

FRANK FENNER

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Introduction

In spite of persistent and laborious investigations for more than fifty years, the mechanism of immunity in tuberculosis is still ill understood. The failure of the direct attack on the problem justifies the use of model systems which avoid some of the complications present in the study of tuberculosis in the experimental animal. Infection of mice by footpad inoculation with *Mycobacterium balnei* and *Mycobacterium ulcerans* is one such model (1). The lesions can be initiated with a very small inoculum and are localized to the inoculated foot, which can be observed throughout the course of the disease; and the progress of infection with *M. balnei* is so rapid that infection-immunity can, if necessary, be avoided.

As the first part of an investigation into immunity in tuberculosis using this model, an examination has been made of the protection against symptoms due to *M. balnei* and *M. ulcerans* conferred on mice by prior infection with the same or other species of mycobacteria. The results reported here demonstrate the existence of a high degree of homologous and a varying degree of heterologous protection, as well as the presence in certain cases of a tuberculin type of sensitization. It was not possible to transfer protection passively with serum from actively immunized animals.

Materials

Bacterial strains: The bacterial strains used in these experiments have been described previously (1). In addition, some experiments were carried out with "Branch," a virulent strain of *M. tuberculosis* var. *bovis*, kindly provided by Dr. G. B. Mackaness. PPD prepared from *M. balnei* by trichloroacetic acid treatment of the filtrate of killed culture was kindly supplied by Mr. A. B. Paterson, Weybridge Laboratories, England.

Culture media: As in previous reports from this laboratory (1), Tween®-albumin liquid medium was used for the routine culture of the mycobacteria and the solid oleic-acid-albumin-agar medium for viable counts. Cultures of *M. balnei* and *M. ulcerans* were incubated at 33°C.; other species, at 37°C.

Experimental animals: Mice were bred in the University Animal Breeding Establishment, and were derived from a multicolored line obtained from the Walter & Eliza Hall Institute of Medical Research, Melbourne, in 1951. Experiments still in progress have shown that several pure-line strains of mice react quite differently from these animals in response to footpad inoculation with *M. balnei*. Some strains develop generalized infection, and in others swelling of the foot is scarcely detectable even after the inoculation of large doses.

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Methods

The development of immunity to reinfection with *M. ulcerans* or *M. balnei* could be satisfactorily studied only after initial inoculation into a footpad, for only thus could the development of lesions at the site of the second inoculation be presumed to be due to that inoculation, and not to the development of a secondary lesion consequent upon an intravenous inoculation. With *M. balnei*, generalized disease sometimes occurred after pad inoculation (4 of 24 maintained for sixteen weeks after inoculation of a large dose of bacilli into the footpad (1)). Nevertheless, in any group of 6 mice it would be extremely unlikely for secondary lesions to occur only in the second inoculated foot of all mice of the group. With other species of mycobacteria, such as BCG, *M. ranae*, and *M. phlei*, the initial inoculation was made both intravenously and by footpad inoculation. The general plan of the experiments to be described was to inoculate six- to eight-week-old mice in one hindfoot, or intravenously, with a concentrated suspension of one species of acid-fast bacilli, and five to seven weeks later to carry out a challenge inoculation with *M. ulcerans* or *M. balnei* in the other footpad. Challenge infection with virulent mammalian tubercle bacilli was carried out by intravenous inoculation. At daily or weekly intervals after the challenge inoculation the mice were examined, and the degree of swelling of the last inoculated footpad was graded according to an arbitrary scale of severity. In some experiments the anteroposterior diameter of the foot was measured to the nearest half millimeter with sliding calipers, and the degree of swelling was expressed as the difference between this measurement and that of the contralateral normal foot. Comparison of the curves obtained with these measurements and with the arbitrary scales indicated close agreement between the two methods.

Experimental Results

Experiments with M. Balnei

The dosage of the challenge inoculation: Mice were inoculated in one hind footpad with 10^6 viable units of *M. balnei* strain K_2O. Seven weeks later groups of 6 mice were challenged by inoculation in the other hindfoot, doses of the same microorganism ranging in tenfold dilutions from $10^{6.6}$ to $10^{0.6}$ viable bacterial units. Control mice were inoculated in one foot with saline at the time of the immunizing inoculation and challenged in parallel with the test mice. The results are shown graphically in figure 1.

Swelling of the inoculated feet of the control mice occurred with all dosages and increased at about the same rate whatever the dosage. As shown previously (1), the swelling commenced about a day later with each tenfold dilution. With the largest challenge inoculum (6.6 R of figure 1) there was an initial accelerated response, and the inoculated foot then increased slowly in size. An accelerated response also occurred with the next largest dose ($10^{5.6}$ viable units—5.6 R), but after the second day the inoculated feet gradually diminished in size, whereas those of the control mice increased progressively to reach the maximal attainable

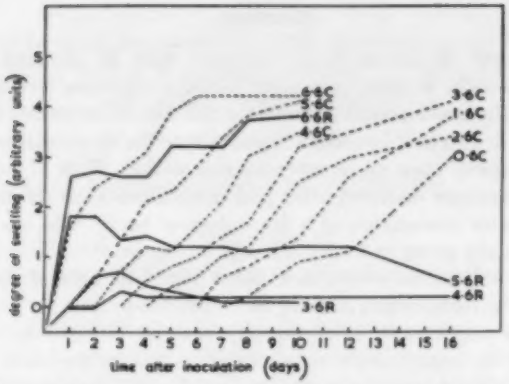

FIG. 1. The course of development of the swelling in the second inoculated foot following the inoculation of 10^6 viable units of *M. balnei*, or of normal saline, into the other foot seven weeks earlier. Broken lines indicate the response of control mice previously inoculated with saline; solid lines, the response of mice previously inoculated with *M. balnei*. 6.6C to 0.6C = doses of $10^{6.6}$ to $10^{0.6}$ viable bacterial units of *M. balnei*, inoculated into control (saline) mice. 6.6R to 3.6R = doses of $10^{6.6}$ to $10^{3.6}$ viable bacterial units of *M. balnei* inoculated into test (*M. balnei*-inoculated) mice. Reinoculation of doses less than $10^{3.6}$ viable bacterial units failed to cause swelling of the foot.

size. In the vaccinated mice there was a slight response only to challenge infection with $10^{4.6}$ viable units, and with smaller doses there was virtually no change in the inoculated foot. The general picture with the large challenge doses was initially an accelerated response, maximal twenty-four to forty-eight hours after inoculation, followed by a gradual increase in size presumably due to multiplication of the bacilli, or a gradual decrease in the size of the foot. When the dose was further reduced the accelerated response did not occur, and in the immunized animals multiplication of the bacilli did not reach the level required to produce obvious swelling of the foot. Thus, inoculation with *M. balnei* seven weeks before challenge infection with the same organism induced a state of allergy, which could be demonstrated by the inoculation of doses of bacilli larger than $10^{4.6}$ viable units, and a state of immunity which completely protected the mice against doses smaller than $10^{4.6}$ viable units.

The interval between immunizing and challenge inoculation: Seven groups each of 6 male mice were inoculated in the left hind footpad with 10^6 viable bacterial units of *M. balnei* strain K_2O. Three days later, and then at weekly intervals for six weeks, one group of these mice and 6 uninoculated animals of the same age and sex were inoculated in the right hindfoot pad with $10^{6.6}$ viable bacterial units of the same strain, contained in 0.05 ml. of fluid. The size of each foot was determined daily by inspection and by measurement of the anteroposterior diameter with a sliding caliper. The results are recorded graphically in figure 2.

The swelling of the feet of the control mice followed the same course each week and was similar to that observed in many other experiments. Prior infection in

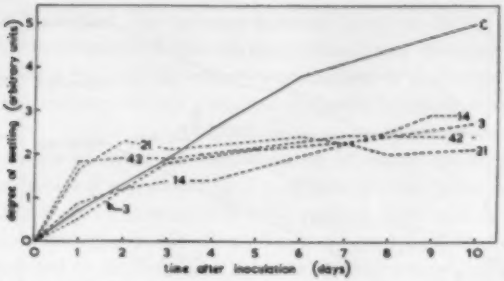

FIG. 2. The alteration in the response of mice inoculated in one foot with *M. balnei* to reinoculation with a large dose ($10^{6.6}$ viable bacterial units) of the same strain in the other foot at intervals varying from three to forty-two days later. Solid line (*C*) = response of control mice (composite of seven experiments). Broken lines = responses of mice reinoculated at the intervals indicated (days).

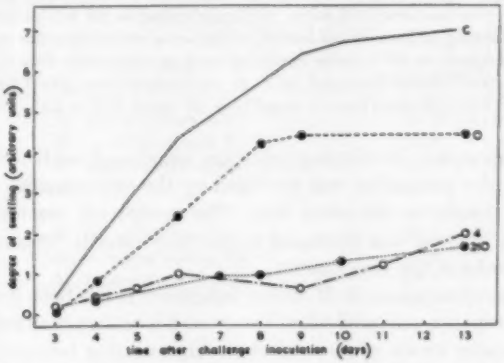

FIG. 3. The alteration in the response of mice inoculated in one foot with *M. balnei* to reinoculation with a small dose ($10^{3.6}$ viable bacterial units) of the same strain in the other foot at intervals varying from 0 to 210 days later. The results of experiments at intervals intermediate between 4 and 210 days are omitted, as they did not differ significantly from these. Solid line (*C*) = response of control mice (composite of ten experiments). Broken lines = responses of mice reinoculated at the intervals indicated (days).

one foot with *M. balnei*, even three days before the challenge infection, modified the response of mice to the challenge inoculation. With the large challenge dose employed in this experiment there was no great increase in the degree of resistance demonstrable at increasing intervals between the first and second inoculations. An accelerated response of tuberculin type occurred twenty-one days after the first inoculation, and remained about the same throughout the experiment. The accelerated response was unchanged twenty-four weeks after the sensitizing inoculation.

In figure 3 are shown the results of a similar experiment in which reinoculations were carried out with small doses of bacilli ($10^{3.6}$ viable bacterial units).

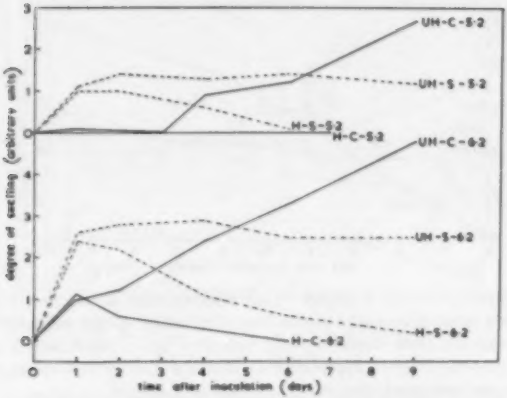

FIG. 4. The response of mice inoculated in one foot with 10^5 viable bacterial units of *M. balnei*, and control uninoculated mice, to the inoculation six weeks later of large and moderate doses of living or heat-killed bacilli of the same strain into the other foot. Large dose (lower part of figure) = $10^{6.2}$ viable bacterial units or equivalent dose killed by heating. Moderate dose = $10^{5.2}$ viable bacterial units or equivalent dose killed by heating. Solid lines = control (*C*) mice. Broken lines = sensitized (*S*) mice. *UH* = unheated. *H* = heated.

With the more slowly developing infection associated with the dose of $10^{3.6}$ bacterial units some protection was provided by the simultaneous inoculation of a large dose of bacilli in the other foot. The protection reached its maximal extent when the interval was increased to four days and it remained unchanged for the thirty weeks of the experiment.

Tuberculin type of responses in M. balnei infections: It has been pointed out that an accelerated response occurred when the second inoculum was sufficiently large and was given three weeks or more after the immunizing inoculation. The next experiment was designed to determine whether killed *M. balnei* could elicit this response. Groups of 6 mice were prepared by the inoculation in the right hind footpad of 10^5 viable units of *M. balnei* strain K_2O. Six weeks later these mice, and appropriate control animals, were inoculated in the other hind foot with suspensions containing $10^{6.2}$ and $10^{5.2}$ viable bacterial units of the same strain of mycobacteria, or similar suspensions which had been heated at 65°C. for an hour. The results are shown in figure 4. Both doses of heat-killed bacilli elicited a tuberculin type of response, but the swelling did not persist for more than a few days. With the unheated preparation there was, as in previous experiments, an accelerated response, and the swollen feet remained much the same size for a considerable length of time. This was presumably due to a limited degree of multiplication due to the large dose inoculated.

PPD prepared from *M. balnei*, inoculated in very large doses (0.2 mg.), elicited a mild response in immunized mice, maximal twenty-four to forty-eight hours after inoculation. There was only slight and transient swelling of the feet in control animals. However, the accelerated response elicited with this PPD was much

less pronounced than that due to large doses of killed or living microorganisms. Participation of constituents of the culture medium was excluded by using bacilli grown on a synthetic medium for either sensitization or challenge inoculation and a Tween-albumin culture for the corresponding challenge or sensitization inoculations.

Protection from the effects of the inoculation of M. balnei induced by prior inoculation with other species of mycobacteria: The degree of cross protection to *M. balnei* infection afforded by prior inoculation with various strains of mycobacteria was examined. In the first experiment (*Experiment A*) groups of 10 six-week-old male mice were inoculated in the left hind foot, or intravenously, with *M. balnei*, *M. ulcerans*, and *M. tuberculosis* strain BCG. They were challenged five weeks later. The dosages employed and routes of inoculation used are shown in tables 1 and 2.

With the larger challenge dose of *M. balnei* there were no appreciable differ-

TABLE 1

DOSAGES AND ROUTES OF INOCULATION OF "IMMUNIZING" INOCULA IN CROSS-PROTECTION TESTS

Experiment	Strain	Dose (Viable Units)	Route of Inoculation
A	*M. balnei* strain K_3O	$10^{6.3}$	Pad
	M. ulcerans strain RT	$10^{5.5}$	Pad
	M. tuberculosis strain BCG	$10^{6.0}$	Pad
		$10^{6.3}$	Intravenous
B	*M. balnei* strain K_3O	$10^{6.6}$	Pad
	M. ulcerans strain RT	$10^{5.0}$	Pad
	M. tuberculosis strain BCG	$10^{6.7}$	Pad
		$10^{5.0}$	Intravenous
	M. avium	$10^{6.7}$	Pad
		$10^{7.0}$	Intravenous
	M. phlei	$10^{6.7}$	Pad
		$10^{7.0}$	Intravenous
	M. ranae	$10^{6.3}$	Pad
		$10^{6.6}$	Intravenous

TABLE 2

DOSAGES AND ROUTES OF INOCULATION OF CHALLENGING INOCULA IN CROSS-PROTECTION TESTS

Experiment	Strain	Dose (Viable Units)		Route of Inoculation
		Large	Small	
A	*M. balnei* strain K_3O	$10^{7.5}$	$10^{5.5}$	Pad
	M. ulcerans strain RT	$10^{6.3}$	$10^{4.3}$	Pad
	M. tuberculosis strain "Branch"	$10^{6.3}$		Intravenous
B	*M. balnei* strain K_3O	$10^{6.3}$	$10^{4.3}$	Pad
	M. tuberculosis strain "Branch"	$10^{6.3}$		Intravenous

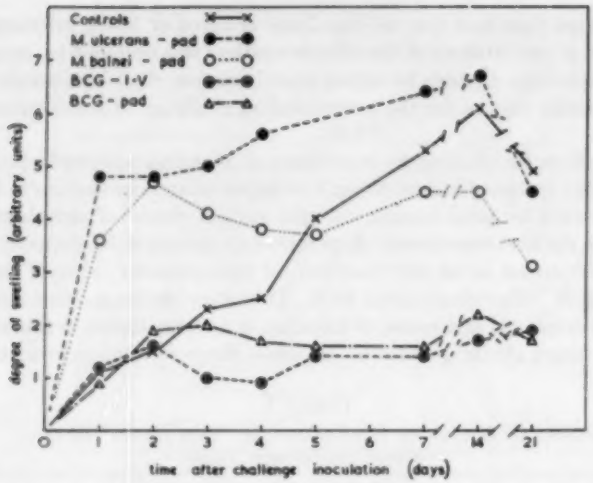

FIG. 5. The effect of prior inoculation with *M. ulcerans*, *M. balnei*, and BCG five weeks earlier on the response of mice to the inoculation of $10^{5.5}$ viable units of *M. balnei* into the right footpad (*Experiment A*).

ences between the controls and the immunized mice. The results with the smaller dose, shown in figure 5, indicate that prior inoculation with *M. balnei* and *M. ulcerans* induced allergy to *M. balnei*, and some protection was evident in mice which had been inoculated with *M. balnei*. No accelerated response occurred after prior inoculation with BCG, but there was an appreciable degree of protection.

In the second experiment (*Experiment B*) tests were made with a larger variety of species of mycobacteria. Observation was confined to an examination of the feet on the third, seventh, eleventh, and fourteenth days in animals challenged with the larger dose of *M. balnei*, and the sixth, fourteenth, and twentieth days in animals challenged with the smaller dose. Evidence of an accelerated response was not sought, and the results are recorded in table 3 in terms of the degree of protection observed. With the larger challenge dose of *M. balnei*, appreciable protection was afforded only by *M. balnei* and BCG inoculated intravenously, and slight protection by BCG and *M. avium* inoculated in the footpad. With the smaller challenge dose, a high degree of protection was evident following vaccination of the mice with *M. balnei*, BCG, or *M. avium*. Some protection was found with *M. ulcerans*, but none at all with *M. ranae* and *M. phlei*.

Protection against M. ulcerans conferred by prior inoculation with various mycobacteria: Owing to the prolonged period necessary for carrying out experiments with *M. ulcerans*, immune phenomena have not been studied as extensively with this species as with *M. balnei*. However, groups of mice prepared in accordance with the schedule of *Experiment A* (table 1) were challenged with *M. ulcerans*. The results obtained are shown in figures 6 and 7. Prior infection with *M. ulcerans*

TABLE 3

PROTECTION AGAINST M. BALNEI AFFORDED BY INOCULATION WITH VARIOUS SPECIES OF MYCOBACTERIA SEVEN WEEKS EARLIER (EXPERIMENT B)

Strain Used for Immunizing Inoculation	Degree of Protection Against *M. Balnei*	
	$10^{6.3}$ Viable Units	$10^{5.3}$ Viable Units
M. balnei	Moderate	Considerable
M. ulcerans	Nil	Slight
M. tuberculosis strain BCG:		
Pad inoculation	Slight	Considerable
Intravenous inoculation	Moderate	Considerable
M. avium:		
Pad inoculation	Slight	Considerable
Intravenous inoculation	Nil	Nil
M. ranae:		
Pad inoculation	Nil	Nil
Intravenous inoculation	Nil	Nil
M. phlei:		
Pad inoculation	Nil	Nil
Intravenous inoculation	Nil	Nil

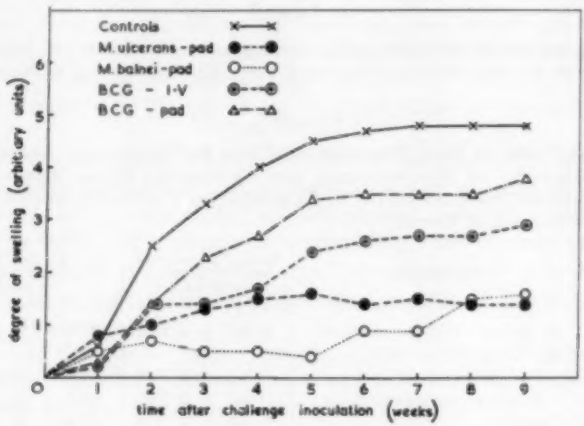

FIG. 6. The response of mice previously inoculated with *M. ulcerans*, *M. balnei*, or BCG to inoculation in the right footpad with a large dose ($10^{6.3}$ viable units) of *M. ulcerans* five weeks later.

or *M. balnei* conferred a high degree of protection against challenge infection with *M. ulcerans*, and BCG gave considerable protection against a small dose and slight protection against a large dose of *M. ulcerans*.

Protection against virulent mammalian tubercle bacilli conferred by prior inoculation with various mycobacteria: Groups of mice prepared as indicated in table 1 were challenged by the intravenous inoculation of *M. tuberculosis* var. *bovis* strain "Branch." Specific death was taken as the end point. In table 4 the sur-

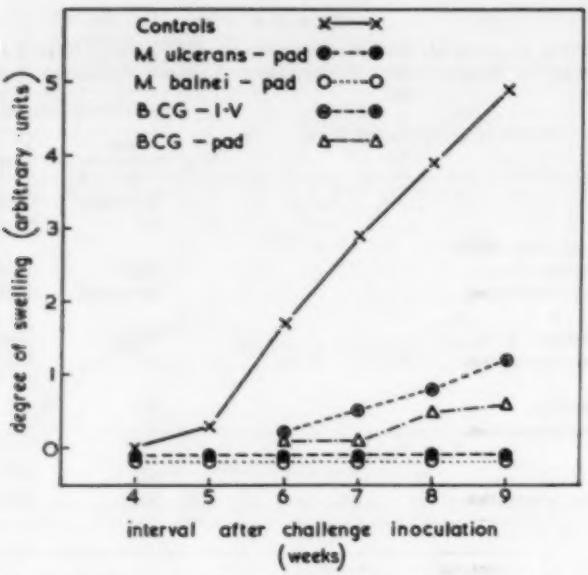

FIG. 7. The response of mice previously inoculated with *M. ulcerans*, *M. balnei*, or BCG to inoculation in the right footpad with a small dose ($10^{4.3}$ viable units) of *M. ulcerans* five weeks later.

TABLE 4

THE SURVIVAL TIMES OF MICE PREPARED BY PRIOR INOCULATION OF LARGE DOSES OF DIFFERENT SPECIES OF MYCOBACTERIA, AND CHALLENGED SEVEN WEEKS LATER BY THE INTRAVENOUS INOCULATION OF M. TUBERCULOSIS VAR. BOVIS STRAIN "BRANCH" (10^6 VIABLE BACTERIAL UNITS)

Experiment Number	Pre-Treatment	Mean of Transformed Survival Times* ± Standard Error	Mean Survival Time in Days and Its 95 Per Cent Fiducial Range		
1	*M. balnei:* Pad inoculation	0.865 ± 0.1975	22.9	27.3	33.4
	M. ranae:				
	Pad inoculation	0.944 ± 0.1260	25.0	28.8	33.2
	Intravenous inoculation	0.645 ± 0.1863	22.7	24.4	73.0
	Controls	0.728 ± 0.1116	21.4	21.5	59.0
2	*M. avium:*				
	Pad inoculation	1.005 ± 0.1527	21.4	30.1	91.9
	Intravenous inoculation	1.046 ± 0.1404	21.8	31.1	87.5
	Controls	0.514 ± 0.0760	21.2	23.6	32.4
3	BCG:				
	Pad inoculation	1.461 ± 0.0701	31.7	48.9	91.1
	Intravenous inoculation	1.320 ± 0.1807	22.2	40.9	214.5
	M. ulcerans: Pad inoculation	1.107 ± 0.1403	22.1	32.8	97.6
	Controls	0.968 ± 0.0739	22.6	29.3	53.6

* Transformed survival time = log(survival time in days—20).

TABLE 5

COMPARISON OF TRANSFORMED MEAN SURVIVAL TIMES OF "IMMUNIZED" AND CONTROL MICE

Statistical Analysis of Data of Table 4. All Comparisons in Each Experiment Made with the Appropriate Control Group

Experiment Number	Pre-Treatment	Degrees of Freedom	t	P
1	*M. balnei:* Pad inoculation	28	0.6103	>0.50
	M. ranae:			
	Pad inoculation	28	1.1888	>0.20
	Intravenous inoculation	28	0.4046	>0.50
2	*M. avium:*			
	Pad inoculation	26	3.2150	<0.01
	Intravenous inoculation	26	3.6120	<0.01
3	BCG:			
	Pad inoculation	26	4.1670	<0.01
	Intravenous inoculation	25	2.9150	<0.01
	M. ulcerans: Pad inoculation	26	0.9660	>0.30

vival times of mice in the different groups have been transformed by applying the metameter $y = (u - u_0)^i$ suggested by Kapteyn (2). Since no mouse died of tuberculosis before the twentieth day, u_0 was taken as twenty days. The survival time thus became (u − 20) days, and a log transformation was carried out on the data.

Comparisons were made between the transformed mean survival times of each group, using the appropriate controls in each instance, and employing the t test. The values of t are shown in table 5 and indicate that under the conditions of these experiments BCG had a significant immunizing effect against infection with virulent tubercle bacilli, a slight but significant protection was conferred by *M. avium*, but no protection followed prior infection with *M. ranae*, *M. phlei*, *M. balnei*, or *M. ulcerans*.

Attempts to demonstrate passive transfer of immunity to M. balnei infections: The difficulties inherent in designing experiments to demonstrate passive transfer of immunity to tuberculosis have been enumerated by Rich (3). As several of these difficulties could be avoided in experiments involving challenge infection of mice with small doses of *M. balnei* inoculated in the footpad, attempts were made to effect passive transfer of protection.

A large group of mice was prepared by the inoculation of large doses of *M. balnei* into the four feet, and a smaller group was inoculated in one hind foot only and kept as actively immunized control animals. Three, four, five, and six weeks after inoculation into all four feet, groups of about 200 mice were bled out, their sera separated and filtered through a Seitz pad. The sera were pooled. A pool of normal mouse serum was also prepared. For one day before challenge inoculation, and daily thereafter for fourteen days, groups of 4 mice were inoculated intraperitoneally with 1 ml. of normal or immune serum. These groups, additional

groups of actively immunized control mice, and groups of normal mice were challenged by the inoculation of 10 and 100 viable bacterial units of *M. balnei*. The actively immunized mice were completely protected, but the feet of the mice inoculated with immune serum could not be distinguished from those of controls inoculated with normal serum. Thus, even large doses of immune serum inoculated daily throughout the course of infection of mice challenged with very small doses of *M. balnei* failed to influence the course of the infection, although actively immunized control animals were completely protected.

Discussion

The rapidity with which mice responded to the footpad inoculation of small doses of *M. balnei*, and the accessibility of the site for daily examination, suggested that this model might be useful in studying immunity in mycobacterial infections. It was possible to demonstrate active immunity to small doses of *M. balnei* after prior inoculation of the homologous organism a few days or many weeks previously. Moreover, a tuberculin type of sensitization could be elicited by large doses of the organism (or with a PPD prepared from it) when the interval between preparation and challenge was three weeks or longer. It will be recalled that Pagel (4) demonstrated the accelerated development of skin lesions in mice inoculated intracutaneously a second or third time with tubercle bacilli. More recently, Gray and Jennings (5) were able to demonstrate a tuberculin type of sensitivity in mice sensitized with BCG and challenged with tubercle bacilli or tuberculin inoculated into the footpad. Selbie and O'Grady (6) demonstrated hypersensitivity and immunity by inoculation of tubercle bacilli into the thigh muscles of mice.

The results illustrated in figure 5 were confirmed in other experiments of the same nature. Prior inoculation with BCG conferred a high degree of protection but no sensitization to a dose of *M. balnei* which elicited an accelerated response in mice immunized with *M. balnei* or *M. ulcerans*. Although the mouse is not a very satisfactory animal for studies on tuberculin sensitivity, the immunization of mice by the intravenous injection of BCG permitted satisfactory comparative observation of normal and inoculated hind feet. This clear-cut dissociation between hypersensitivity and immunity may have wider importance in the study of the mechanism of immunity in mycobacterial infections.

It proved impossible to influence the course of infection of mice with very small doses of *M. balnei* by daily inoculation with large doses of homologous immune serum. The design of this experiment, in which large doses of homologous immune serum were inoculated daily throughout the course of the infection of mice with small doses of *M. balnei*, avoided most of the difficulties encountered in attempts to transfer passive immunity to *M. tuberculosis*. Consequently, the negative result must be accepted as strong evidence that immunity in mycobacterial infections cannot be passively transferred with serum.

The cross-protection evident with both *M. balnei* and *M. ulcerans* and other mycobacteria contrasts with the failure to demonstrate heterologous protection against challenge with virulent tubercle bacilli, both in the current experiments

and in the experience of other workers (7). Footpad inoculation with small doses of bacilli is probably a much more sensitive test of immunity than can be employed in infections with virulent tubercle bacilli.

The protection against both *M. balnei* and *M. ulcerans* conferred by immunization with BCG may have some practical importance, for infection of man with these organisms, although rare, may sometimes be troublesome.

Summary

Footpad inoculation with *M. balnei* and *M. ulcerans* was used to study homologous and heterologous immunity in mice infected with a variety of mycobacteria. The shorter incubation period in *M. balnei* infections made these more satisfactory experimental models. Homologous immunity was demonstrable with increasing effectiveness as the dosage of the challenge inoculation was diminished. With large challenge inocula some protection occurred at an interval of three days between infection and challenge, but a tuberculin type of sensitization was not demonstrable until the interval was increased to twenty-one days. A high degree of protection was evident after four days with small challenge inocula, and persisted unchanged for at least thirty weeks.

Heterologous protection was demonstrated with both organisms. *M. ulcerans*, *M. balnei*, and BCG all induced a high degree of protection against challenge infection with small doses of *M. ulcerans*. *M. balnei*, *M. avium*, and BCG produced substantial protection against challenge infection with small doses of *M. balnei*. Protection induced by *M. ulcerans* and *M. balnei* was associated with an accelerated reaction to the challenge inoculum, but mice immunized with BCG showed a high degree of protection without any evidence of sensitization. Prior inoculation with *M. ranae* and *M. phlei* failed to immunize mice to challenge infection with *M. balnei*.

Attempts to effect passive transfer of protection against infection with very small doses of *M. balnei* by daily inoculation with serum from mice actively immunized with *M. balnei* were unsuccessful.

Prior infection with *M. ulcerans* or *M. balnei* failed to prolong the survival time of mice challenged by the intravenous inoculation of virulent tubercle bacilli, although both BCG and *M. avium* produced significant prolongation of the survival time.

Sumario

La Inmunidad Homóloga y Heteróloga en las Infecciones Murinas con Mycobacterium Ulcerans y Mycobacterium Balnei

Para estudiar la inmunidad homóloga y heteróloga en los ratones infectados con una forma de micobacterias se utilizó la inoculación en la planta del pie. El período de incubación más corto en las infecciones por *M. balnei* hizo que éstas fueren más satisfactorias como modelos de experimentación. A medida que disminuía la dosis de la inoculación provocadora se descubría con mayor efectividad la inmunidad homóloga. Con grandes inóculos provocadores ya había alguna protección en un plazo de tres días entre la infección y la provocación, pero no se podía observar una forma tuberculínica de sensibilización hasta que el plazo subía a veintiún días. Con los pequeños inóculos provocadores se evidenciaba protección

de alto grado al cabo de cuatro días, continuando inalterada a lo menos durante treinta semanas.

Se observó protección heteróloga con ambos gérmenes. El *M. ulcerans*, el *M. balnei* y el BCG produjeron por igual protección de alto grado contra la infección provocada con pequeñas dosis de *M. ulcerans*. El *M. balnei*, el *M. avium* y el BCG produjeron protección substancial contra la infección provodada con pequeñas dosis de *M. balnei*.

La protección inducida por el *M. ulcerans* y el *M. balnei* se vinculó con reacción acelerada al inóculo provocador, pero los ratones inmunizados con BCG revelaron un tenor alto de protección sin signo alguno de sensibilización. La inoculación anterior con *M. ranae* y *M. phlei* no inmunizó a los ratones a la infección provocada con *M. balnei*.

No dieron resultado los esfuerzos realizados para lograr el pase pasivo de protección contra la infección con pequeñas dosis de *M. balnei* mediante la inoculación diaria de suero procedente de ratones inmunizados con *M. balnei*.

La infección anterior con *M. ulcerans* o *M. balnei* no prolongó el tiempo de sobrevivencia de los ratones provocados con la inoculación endovenosa de los bacilos tuberculosos virulentos, aunque tanto el BCG como el *M. avium* produjeron una prolongación importante del tiempo de sobrevivencia.

Resume

L'immunité homologue et hétérologue dans les infections de la souris à Mycobacterium Ulcerans et à Mycobacterium balnei

Chez la souris, l'inoculation de la plante de la patte avec le *M. balnei* ou avec le *M. ulcérans* a été utilisée pour étudier l'immunité homologue ou hétérologue des souris inoculées avec diverses mycobactéries. La période d'incubation plus courte de l'infection à *M. balnei* rend celle-ci plus satisfaisante comme type expérimental. Plus la dose de l'inoculation-test était diminuée plus la démonstration de l'immunité homologue était facilitée. Lorsque la dose de réinoculation-test était importante, il se produisait un certain degré d'immunité au bout de trois jours entre l'infection et la réinoculation-test mais l'allergie du type tuberculine n'était observée qu'au bout d'un intervalle de vingt et un jours. La démonstration d'un degré d'immunité élevé était obtenue au bout de quatre jours avec des doses faibles d'inoculum-d'épreuve, et cette immunité était maintenue sans changement pendant au moins trente semaines.

L'immunité hétérologue a été démontrée avec ces deux mycobactéries. *M. ulcerans*, *M. balnei* et BCG ont tous provoqué un degré d'immunité élevé contre la réinfection test avec de petites doses de *M. ulcerans*. Le *M. balnei*, *M. avium* et BCG entraînaient une immunité substantielle contre la réinoculation-test avec de petites doses de *M. balnei*.

L'immunité acquise par l'inoculation de *M. ulcerans* et *M. balnei* s'accompagnait d'une réaction accélérée à la réinoculation-test d'épreuve, mais les souris immunisées par le BCG révélaient un degré d'immunité élevé sans qu'apparaisse aucun signe de sensibilisation. L'inoculation préalable avec le *M. ranae* et *M. phlei* n'immunisait pas les souris contre la réinoculation-test avec le *M. balnei*.

Des tentatives pour effectuer le transfert passif de l'immunité avec de petites doses de *M. balnei* par l'inoculation quotidienne de sérum de souris immunisées avec *M. balnei* ont échoué. L'infection préalable avec *M. ulcerans* ou *M. balnei* n'a pas réussi à augmenter le temps de survie des souris soumises à l'inoculation intraveineuse de bacille tuberculeux virulent; toutefois le BCG et le *M. avium* ont produit une prolongation significative du temps de survie.

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THE ROLE OF HUMORAL FACTORS IN NATIVE AND ACQUIRED RESISTANCE TO TUBERCULOSIS[1]

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INTRODUCTION

In general, phagocytosis has been considered one of the most important factors in the defensive power of living organisms against various infectious diseases. Although in tuberculosis, too, the functions of phagocytic cells are thought to be a very important factor in native resistance, it has not yet been satisfactorily proved that these phagocytic cells are able to kill the phagocytized bacilli or to restrict the proliferation of bacilli. Moreover, investigators (1) have reported that virulent tubercle bacilli may be able to survive or even to multiply in these phagocytic cells. Recently Suter (2) and Mackaness and associates (3) proved definitely by *in vitro* experiments that virulent tubercle bacilli, including BCG, could proliferate in mononuclear cells of normal rabbits and guinea pigs. Thus, it is reasonable to suppose that phagocytosis may not play an exclusive role in the native resistance of living organisms against virulent tubercle bacilli. Another factor, the humoral factor, must be investigated.

In acquired resistance to tuberculosis, the role played by the functioning of phagocytic cells has likewise not been clearly determined. Lurie (4) found that in acquired resistance both cellular and humoral functions had increased their power and that the role of cellular functions might be superior. Recently Suter (5) found that intracellular growth of virulent tubercle bacilli *in vitro* was apparently inhibited by immunization. Using somewhat different techniques, Mackaness (6) was not able to reproduce Suter's results and he found that the intracellular growth of tubercle bacilli was almost the same in preparations obtained from both immunized and nonimmunized control animals.

It has generally been considered that there is no satisfying proof of the existence of an antimicrobial action of the body fluids in immunity to tuberculosis. Several investigations (7–11), apparently demonstrating a bacteriostatic effect of serum in immunity to tuberculosis, have been reported. However, other investigators (12–14) have been unable to show such a phenomenon. Rich (15) stated that "it must be admitted, however, that satisfying proof of the existence of a special bacteriostatic action of acquired resistance is at present wanting." Therefore, the role of humoral factors must be investigated in acquired as well as in native resistance.

In order to determine the significance of the humoral factors, a special experimental method is needed. Ordinary *in vitro* experiments are not adequate for this purpose because body fluids, such as serum or lymph, may change their characteristics on removal from the body and participate in reactions different from those which take place within the body.

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TABLE 1

THE GROWTH OF VARIOUS STRAINS OF MYCOBACTERIA IN O CHAMBERS IN VARIOUS ANIMAL SPECIES

Animals	Strains						
	Bovine RM	BCG	Human Aoyama *B*	Human H37Rv	Avian Cho-Kyo	*M.* 607	Smegma Bacilli
Rabbit	++	+	++	++	+	−	−
Guinea pig	++	+	++	++	−	−	−
Hamster	−	±	−	−	−	−	−
Rat	−	±	−	−	−	−	−
Hen	−	−	−	−	+	−	−
Dog	+	±		+			

++....Very good proliferation ±....Sometimes slight multiplication and sometimes no growth

+....Good proliferation −....No bacillary growth

The present paper consists of a report of findings of *in vivo* and *in vitro* experiments, using newly devised methods, on the role of the humoral factors in the immune response to tuberculous infection.

METHODS

The experimental method (the chamber method) employed has been reported and the technique thoroughly described in a previous publication (16). The principle of this method is as follows:

A chamber consisting of a membrane filter and a watch-glass is made. Inside of this watch-glass, bacilli, distributed as a single bacillus by the Benzine®, [2] method, are smeared and fixed. This chamber is surgically implanted in the peritoneal cavity of the experimental animal after being filled with various materials. In the peritoneal cavity, body fluid is allowed to pass through the filtering membrane, but no cells can enter the chamber. Bacilli are thus cultivated by a type of slide culture method in a medium of body fluid completely separated from cells.

Three different chambers were used, with changes of the filtering membranes and the contents of the chambers.

According to the type of filtering membrane and the contents of the chamber, the content of the chamber after the implanting (culture medium for the smeared bacilli) varies in each chamber as shown in table 2.

OBSERVATIONS

Experiments Related to Native Resistance

Experimental results, using the *O* chambers to observe the growth of various mycobacteria in the peritoneal fluids of various animal species *in vivo*, are shown in table 1. These data may be interpreted as follows:

The initial growth rates of various strains in the body fluids of normal animals almost paralleled the virulence of these strains to the corresponding animal species. Nonpathogenic mycobacteria were entirely unable to multiply in the body fluids. Virulent tubercle bacilli (bovine and human strains) grew well in the body fluids of

[2] The writers made the mistake of writing benzene instead of Benzine®—petroleum benzinum—in this previous report.

susceptible animals. The attenuated strain BCG, however, also proliferated well in rabbits and guinea pigs although these hosts were ultimately resistant to this strain. Moreover, no difference was observed between the growth rates of the very highly virulent bovine RM strain and the very slightly virulent human strain Aoyama *B* in rabbits. These facts suggest that the speed and efficacy with which the host develops acquired resistance are also important in the determination of virulence. The fact that virulent bacilli cannot grow in hamsters and rats, which have relatively low susceptibilities to these strains, is interesting and should be elucidated in future investigations.

TABLE 2

GROWTH OF VARIOUS MYCOBACTERIA IN CHAMBERS O, S, AND K

Chamber			O	S	K
Filter membrane			Crude parchment paper	Cellophane membrane (No. 300)	Cellophane membrane (No. 300)
Contents of chamber before the implanting			Physiologic saline solution	Physiologic saline solution	10% serum-Kirchner medium
Fraction passing through the filter membrane			Higher and lower molecular fraction	Lower molecular fraction	Lower molecular fraction
Contents of chamber after the implanting			Body fluid separated from cells	Lower molecular fraction of body fluid	Lower molecular fraction of body fluid + 10% serum protein
Growth of smeared bacilli	Normal rabbit	Smegma bacilli	−	±	++
		M. 607	−	±	++
		Avian tubercle bacilli	+	+	+
		H37Ra	+	−	±
		BCG	+	−	±
		Human Aoyama *B*, H37Rv	++	−	±
		Bovine RM			
	Immunized rabbit	BCG	−	−	±
		Human tubercle bacilli			
		Bovine RM			

−.....No bacillary growth
±.....Sometimes slight multiplication and sometimes no growth
+.....Good proliferation
++.....Very good proliferation

In order to analyze the role of the humoral factor, separated into higher and lower molecular fractions, in native resistance to tuberculosis, experiments using three chambers (*O*, *K*, and *S*) smeared with the same strain in the same animal at the same time were performed. Rabbits were used. The results are shown in table 2.

These data may be interpreted as follows:

It is very interesting that nonpathogenic mycobacteria such as smegma bacillus and *Mycobacterium* 607 which cannot proliferate in the *O* chamber were able to grow very well in the *K* chamber. These results definitely indicate that the growth-inhibiting function of body fluid against those nonpathogenic mycobacteria is contained in the higher molecular fraction of body fluid, perhaps in the protein fraction.

Virulent tubercle bacilli (bovine RM, H37Rv, Aoyama *B*), including the BCG strain, which were able to grow well in the *O* chamber, were apparently restricted in their growth in the *K* chamber and were entirely unable to multiply in the *S* chamber (figure 1). These results suggest that the lower molecular fraction of body fluid may possess a powerful inhibiting quality against virulent or attenuated tubercle bacilli; but whole body fluid protein may be antagonistic (growth-promoting) to this function of the lower molecular fraction. In the latter instance no inhibition occurs.

The avian strain showed a different behavior from the other virulent strains. The avian Cho-Kyo strain grew well in all three chambers. Both higher and lower molecular fractions of rabbit's body fluid have no growth-inhibiting action on this strain. This difference in the mechanism of pathogenicity may be one reason why the pathologic findings (so-called Yersin type) found in infections caused by avian tubercle bacilli are different from those found in infections caused by other tubercle bacilli.

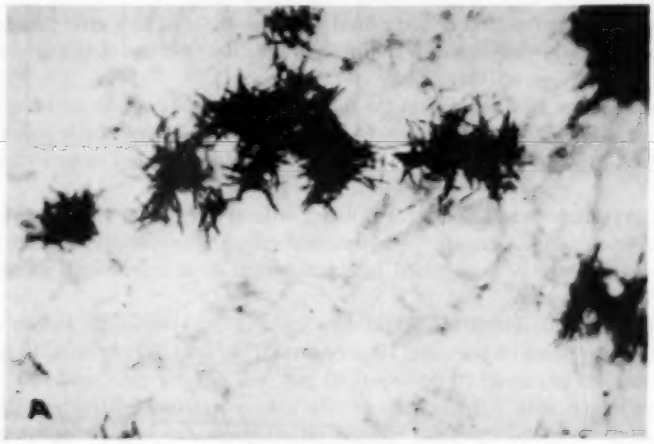

FIG. 1. Colonies of H37Ra strain in the *O* chamber thirty days after implantation into the rabbit's peritoneal cavity.

Experiments Related to Acquired Resistance

Experiments using the chamber method: Rabbits were immunized subcutaneously with 1 mg. of the human strain Aoyama *B* or 50 mg. of the heat-killed bovine strain RM suspended in paraffin oil. Thirty days after the immunizations, the *O*, *K*, and *S* chambers, smeared with the bovine RM strain or the human H37Rv strain, were implanted into the peritoneal cavities of the rabbits. Control experiments were performed on nonimmunized animals. Fifteen to twenty to thirty days after implanting, the growth of bacilli was observed.

In the *O* chambers of control animals, bacillary colonies which could be recognized under low magnification always developed within fifteen to twenty days. However, bacillary growths were apparently inhibited in the immunized animals; sometimes slight proliferations of bacilli were seen under the oil immersion lens, but in most cases no bacillary growth was seen.

In the *K* chambers very slight proliferation, or no proliferation, of bacilli was seen in both immunized and control animals. Bacillary growth was markedly inhibited in the *K* chambers, compared with growth in the *O* chambers. No differences were observed between immunized and normal control animals.

Similar experiments were performed with BCG immunization. One milligram or 10 mg. of BCG strain were inoculated subcutaneously into rabbits. Thirty days after immunization, experiments were performed using the chamber method. The results, summarized in table 2, were almost the same as those of the experiments with the virulent strains above described.

From these experiments it was observed that one month after immunization a powerful tuberculostatic effect developed in the body fluid of rabbits. The results in the *K* chambers were almost the same in both immunized and normal controls. Consequently, this powerful bacteriostatic effect is assumed to have developed mainly in the high molecular fraction of body fluid, perhaps in the protein fraction. It may be questioned whether the growth-promoting function of the high molecular fraction of body fluid has been reduced or a growth-inhibitory function has newly developed in the protein fraction because of the alteration of protein composition of body fluid.

Experiments in vitro to confirm the tuberculostatic action of the serum of immunized animals: An attempt was made to show that the tuberculostatic action which was proved in experiments *in vivo* could also be demonstrated in experiments *in vitro*.

First, experiments were performed using ordinary Kirchner 10 per cent serum-culture medium and the results were determined macroscopically. By this method no difference could be recognized between serum from immunized animals and normal control serum.

Second, the slide culture method was used (17). A series of eleven reagent glasses were prepared: 0 per cent, 10 per cent, 20 per cent, 30 per cent, 40 per cent, 50 per cent, 60 per cent, 70 per cent, 80 per cent, 90 per cent, and 100 per cent sera were mixed with Kirchner liquid (the concentration of Kirchner liquid was varied so as to make the final concentration the same in all reagent glasses). Tubercle bacilli were cultivated in this series of glasses. Bacillary growth was seen easily in glasses containing from 10 to 50 per cent serum. The other glasses showed decreased growth; but even in 90 per cent serum virulent tubercle bacilli

could multiply. Nonpathogenic mycobacteria proliferate even in 100 per cent serum. However no apparent difference was found between bacillary growth in serum from immunized animals and from control serum.

Following this failure, a new experimental method was devised in which serum would be replaced every two days; thus, continuously fresh serum would act on tubercle bacilli. The materials and the procedure used were as follows:

A large metal ring plated with nickel (inner diameter, 1.4 cm; thickness, 0.1 cm.; height, 0.35 cm.).

A smaller metal ring plated with nickel (inner diameter, 1.1 cm.; thickness, 0.1 cm. height, 0.15 cm.).

Glass slides, 7.5 × 2.5 cm.

Cover glasses (3.0 × 2.5 cm.) with a small hole (0.15 cm. in diameter) drilled near the center. This hole is used for replacing the culture medium.

The smaller ring was fixed with paraffin on a glass slide and bacilli suspended in Benzine were smeared on the glass slide inside the ring. After the Benzine dried, the large ring was placed so that the smaller ring was almost in the center of the large ring (figure 2). The

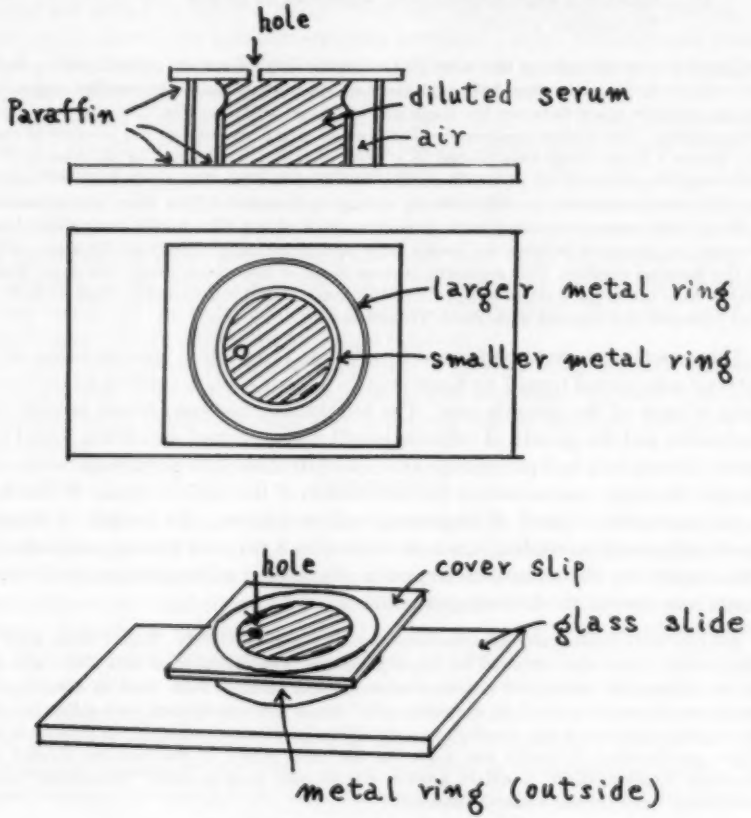

FIG. 2. Diagram of the ring method.

TABLE 3

GROWTH OF TUBERCLE BACILLI IN RABBIT SERUM WHICH IS DILUTED WITH 0.9 PER CENT SALINE SOLUTION OR KIRCHNER MEDIUM

		Incubation in Days			
		7	9	11	13
Serum + saline solution	Nonimmunized	+	+	++	++
	Immunized	−	−	−	
Serum + Kirchner medium	Nonimmunized		+++	+++	+++
	Immunized		++	+++	+++

+++....Colonies adhere to each other, and there are no distinct colonies
++....Very good proliferation, but there are distinct colonies
+....Good proliferation (by oil immersion)
±....Sometimes slight proliferation, sometimes no growth
−....No bacillary growth

preparation was covered and the cover glasses on the ring were made airtight with paraffin. The culture media were poured through the hole into the inside of the smaller ring with a syringe and the space between the rings was left filled with air. The hole was then closed with paraffin. (The culture medium inside the small ring does not flow out because of capillary action.) These rings were placed in a Petri dish and maintained in an oven at 37°C. Forty-eight hours later, an injection needle, sterilized in a gas flame, was inserted into the hole and the contents were withdrawn by syringe and replaced by a new culture medium.

When these procedures are done very gently, the flushing off of bacilli during the change of media is prevented because the bacilli have been fixed fairly tightly on the glass surface by the Benzine method. This replacing is done three or four times every two days. Ten to twelve days after the start of cultivation, the glass slide preparation is fixed with 10 per cent formalin and stained with Ziehl-Neelsen stain.

The experiments were performed in rabbits. The protein concentration of the rabbits' sera varied from 7 to 8 per cent to 4.5 per cent, according to the conditions of each of the animals used. The relationship between serum protein concentration and the growth of tubercle bacilli was examined and it was found that serum diluted to 3 to 5 per cent protein concentration with physiologic saline was always the most convenient for the cultivation of the H37Rv strain. If Kirchner liquid was used in place of physiologic saline solution, the growth of tubercle bacilli improved. Accordingly, serum diluted to 3 per cent protein concentration (determined by the refractometer) with physiologic saline solution or Kirchner liquid was used in the following experiments.

Rabbits were inoculated subcutaneously with 1 mg. of H37Rv. Thirty days after immunization, blood was removed by heart puncture. The serum from this blood was used as the immunized serum. Of course, nonimmunized animals were used as controls. The results are shown in table 3. In normal control serum medium diluted with saline solution, the bacilli proliferated day by day. In serum from immunized animals, no growth or only slight proliferation of bacilli was seen. On the other hand, in the medium diluted with Kirchner liquid medium, bacillary growth was equally good in both "immunized" serum and serum from normal control animals.

TABLE 4

THE EFFECT OF RABBIT SERUM IMMUNIZED WITH HEAT-KILLED BOVINE TUBERCLE BACILLI ON THE GROWTH OF TUBERCLE BACILLI

Immunization	Growth	
	Before Immunization	After Immunization
mg.		
Control	+++	+++
10	+++	++
50	+++	+++
100	+++	±
200	+++	−

Similar experiments were performed by immunizing with a heat-killed bovine RM strain suspended in paraffin oil (10 mg., 50 mg., 100 mg., and 200 mg. intramuscular inoculations). Sera were diluted with physiologic saline solution. The results are shown in table 4. Serum from animals immunized with 200 mg. of dead bacilli showed the best bacteriostatic action and serum from animals immunized with 100 mg. of bacilli was next best. The other groups of sera showed no apparent differences from the sera from control animals.

These results indicate that a normal serum diluted with saline solution does promote growth of tubercle bacilli and a normal serum diluted with Kirchner liquid medium does not prevent growth; but, on the other hand, an immune serum diluted with saline does not promote growth and an immune serum diluted with Kirchner medium does not inhibit growth. Therefore, it seems definite that the bacteriostatic effect developed in the body fluid of an immunized rabbit is attributable to the reduction or the loss of the growth-promoting function contained mainly in the high molecular fraction of body fluid. The exact proof of this inference is still a problem for future solution.

In short, it has been shown that the growth of virulent tubercle bacilli has been interfered with in the body fluids and the sera of immunized rabbits. This may be a proof that humoral factors play a definite role in acquired resistance to tuberculosis.

DISCUSSION

Lurie (4) reported an interesting *in vivo* method of observing the function of body fluids separated from cells—the method of the collodion silk bag. He recognized by this method that body fluids of normal animals could not restrict the multiplication of virulent tubercle bacilli, although body fluids of immunized animals apparently acquired a definite ability to restrict the proliferation of bacilli. Criticism of this finding of Lurie was presented by Rich (15) and Dubos (18), who assumed that this ability of restricting the growth of bacilli in the immunized animals was not the result of specific alteration of characteristics of body fluids by immunization, but was the result of secondary changes in the environment caused by particular tissue reactions in immunized animals. The present writers have found the same results as were found by Lurie by using a

new, although similar, experimental method *in vivo*. By the present methods it was possible to analyze the functions of body fluid in more detail than by Lurie's method. Nevertheless, the same criticism may be made of the present experiments *in vivo* on acquired resistance. It was also possible to demonstrate the existence of bacteriostatic power in the serum of immunized animals *in vitro*. It must be conceded that the pH of the environment was not controlled in either the *in vitro* or the *in vivo* experiments, and this aspect of the question could conceivably be important. Although the question cannot be regarded as settled, it seems to the writers to be more likely that the multiplication of virulent tubercle bacilli is interfered with in the body fluids of immunized animals without the secondary influence of the environment around the chamber on the bacilli.

It is inferred that this bacteriostatic power may have been brought about by the reduction of the growth-promoting function of body fluids and not by the increase of the growth-inhibitory function.

It is generally known that serum albumin is able to promote growth of virulent tubercle bacilli (19). In tuberculous or immunized animals the serum-albumin frequently decreases and the serum-globulin correspondingly increases. This decrease in the albumin fraction may be one reason for the reduction of the growth-promoting function of immunized serum. However, it has not yet been definitely proved that the increased globulin fraction has had no tuberculo-inhibitory power.

With reference to the mechanism of native resistance to tuberculosis, it is believed that several new findings were obtained by the present methods.

The growth of nonpathogenic mycobacteria such as smegma bacillus and *Mycobacterium 607* was suppressed by the protein fraction of body fluid.

The present method has shown that the lower molecular fraction of the body fluid of normal rabbits is definitely able to restrict the growth of virulent tubercle bacilli. This function has also been established by *in vitro* experiments which will be published subsequently. This ability is ordinarily hidden by the antagonism (growth-promoting function) of the protein fraction of body fluid; therefore the body fluid itself is not able to restrict the multiplying of virulent tubercle bacilli. It is assumed that the relationship between the bacteriostatic power of the lower molecular fraction and the growth-promoting action of the higher molecular fraction regulates the native resistance of animals or the virulence of each strain of bacilli.

The chemical properties of these low molecular substances have not yet been established. Recently Björnesjo (20–24) conducted extensive investigations on the tuberculo-inhibitory properties of urine. He reported that the majority of urine specimens from healthy and tuberculous humans possessed tuberculostatic and tuberculocidal properties, and he attempted to identify this substance. It occurred to Myrvik and associates (25) that the properties of the urinary substance of Björnesjo were not unlike those of ascorbic acid and certain of its derivatives. These works of Björnesjo and Myrvik and associates strongly suggest that the substances they found in normal urine are the same or similar substances to those found by the present writers in normal body fluid.

Dubos (26–28) suggested that there might be various substances in the tissues

of normal animals which have tuberculostatic properties. Lactic acid especially, and other organic acids which are intermediate metabolites of tissue cells and accumulate abundantly in tuberculous foci, have powerful bacteriostatic or bacteriocidal effects on tubercle bacilli under certain circumstances. Patnode (29) reported that tissue fatty acids isolated from lungs of normal rabbits inhibited the growth of virulent tubercle bacilli, particularly tubercle bacilli of human origin. The findings described above strongly suggest that it is not surprising to find a tuberculostatic property in the low molecular fraction of the body fluid of normal rabbits.

Summary

In order to determine the significance of the role of humoral factors in native and acquired resistance to tuberculosis, growth experiments of various mycobacteria have been performed by the chamber method and a newly devised *in vitro* experimental method.

The initial growth rates of various strains in the body fluids completely separated from cells of normal animals (the growths in the *O* chamber) almost parallel the virulence of these strains to the corresponding animal species. However, the speed and efficacy with which the host develops acquired resistance also may be important in the determination of virulence.

The growths of nonpathogenic mycobacteria such as smegma bacillus and *Mycobacterium* 607 are supressed completely in the body fluids of animals. This growth-inhibiting function of body fluid against these nonpathogenic mycobacteria is contained in the higher molecular fraction of body fluid, perhaps protein.

Against virulent or attenuated tubercle bacilli the lower molecular fraction of body fluid has a strong inhibiting power. In whole body fluid, however, the protein fraction may be antagonistic (growth promoting) to this function of the lower molecular fraction, and thus no inhibition occurs. The avian strain shows a different behavior in the chambers from other virulent strains in rabbits.

Approximately one month after immunization (by inoculations of living human tubercle bacilli or heat-killed bovine tubercle bacilli suspended in paraffin oil, or living BCG), a powerful tuberculostatic effect develops in the body fluid of rabbits. This tuberculostatic effect is assumed to develop mainly in the high molecular fraction of body fluid.

From the results of the analytic experiments *in vivo* and *in vitro* it may be inferred that the tuberculostatic effect which develops in the body fluid of immunized rabbits is due to the reduction of the growth-promoting power of the higher molecular fraction of body fluid.

The writers could find no difference in the growth-inhibitory function of the lower molecular fraction of body fluid between immunized and nonimmunized normal rabbits.

Sumario

El Papel de los Factores Humorales en la Resistencia Natural y la Adquirida a la Tuberculosis

A fin de determinar la importancia del papel desemepeñado por los factores humorales en la resistencia natural y la adquirida a la tuberculosis, lleváronse a cabo experimentos

relativos a la proliferación de varias microbacterias por el método de la cámara y con una técnica experimental *in vitro*, recién introducida.

Los coeficientes iniciales de proliferación de varias cepas en los humores orgánicos completamente apartados de las células de animales normales (las colonias en la cámara O) casi paralelan la virulencia de dichas cepas para la especie animal correspondiente. Sin embargo, la celeridad y la eficacia con las que el huésped forme resistencia adquirida quizás también revistan importancia en la determinación de la virulencia.

La proliferación de las bacterias anapatógenas, tales como el bacilo del esmegma y el *Mycobacterium* 607, queda absolutamente suprimida en los humores orgánicos de los animales. Esta función humoral de inhibición del desarrollo de esas micobacterias anapatógenas está contenida en la fracción molecular más alta, tal vez la proteína, de los humores orgánicos.

Contra los bacilos tuberculosos virulentos o atenuados la fracción molecular más baja de los humores despliega una poderosa facultad inhibidora, pero, en el humor orgánico integro, la fracción proteínica puede antagonizar (fomentando el desarrollo) dicha función de la fracción molecular más baja, y de ahí, que no haya inhibición. La cepa aviaria muestra en las cámaras un comportamiento distinto del de otras cepas virulentas en los conejos.

Aproximadamente al mes de la inmunización (con inoculaciones de bacilos tuberculosos vivos de tipo humano o de bacilos tuberculosos bovinos matados al calor y suspendidos en aceite de parafina o de de BCG vivos), aparece un poderoso efecto tuberculostático en los humores orgánicos de los conejos. Se presupone que este efecto tuberculostático ha sido creado principalmente en la fracción molecular alta de los humores orgánicos.

A juzgar por los resultados de experimentos analíticos *in vivo* e *in vitro*, cabe inferir que el efecto tuberculostático que aparece en los humores orgánicos de los conejos inmunizados se debe a la reducción de la facultad fomentadora del desarrollo que posee la fracción molecular más alta de los humores.

No se pudo descubrir la menor diferencia en la facultad inhibidora del desarrollo mostrada por la fracción molecular más baja de los humores orgánicos entre los conejos normales inmunizados y sin inmunizar.

Resume

Le rôle du facteur humoral dans la résistance congénitale ou acquise à la tuberculose

De manière à déterminer l'importance du rôle du "facteur humoral" dans la résistance congénitale ou acquise à la tuberculose l'expérimentation du développement de diverses bactéries a été effectuée par la "chamber method" et par une méthode expérimentale *in vitro* récemment élaborée.

Les taux initiaux de développement de diverses souches dans les liquides organiques complètement séparés des cellules d'animaux normaux (les cultures dans la chambre O) sont presque parallèles à la virulence de ces souches pour les espèces animales correspondantes. Toutefois, la rapidité et l'efficience du développement de la résistance acquise chez l'hôte peuvent également être importantes dans la détermination de la virulence.

La croissance des mycobactéries non pathogènes, comme le bacillus smegma et le *Mycobactérium* 607 est complètement supprimée dans les liquides organiques des animaux. Cette fonction inhibitrice du liquide organique contre ces mycobactéries non pathogènes est incluse dans la fraction moléculaire la plus elevée du liquide organique, peut-être dans la protéine.

La fraction moléculaire la plus reduite du liquide organique possède un pouvoir inhibiteur puissant contre les bacilles tuberculeux virulents ou atténués, mais, dans le liquide organique total, la fraction protéique peut-être antagoniste (favorisant la croissance) de cette fonction de la fraction moléculaire la plus reduite et ainsi il n'y a pas d'inhibition. Chez le lapin la souche aviaire montre dans les "chambres" un comportement qui diffère de celui des autres souches virulentes.

Approximativement un mois après l'immunisation (par inoculation de bacilles tuberculeux humains vivants, ou de bacilles bovins tués par la chaleur, suspendus dans l'huile de

paraffine ou par le BCG vivant) un effet tuberculostatique puissant se développe dans le liquide organique des lapins. Cet effet tuberculostatique est considéré comme se produisant principalement dans la fraction moléculaire élevée du liquide organique.

D'après les résultats des expérimentations analytiques *in vivo* et *in vitro* il est permis de supposer que l'effet tuberculostatique qui se développe dans le liquide organique des lapins immunisés est dû à la réduction de l'activité favorisant la croissance qui appartient à la fraction moléculaire la plus élevée du liquide organique.

Les auteurs n'ont pu observer aucune différence dans la fonction inhibitrice de la croissance de la fraction moléculaire la plus basse du liquide organique entre les lapins normaux non immunisés et immunisés.

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ATYPICAL ACID-FAST BACILLI[1, 2, 3]

I. A Cultural Scheme for Rapid Identification

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INTRODUCTION

The problem of the identification and significance of atypical acid-fast bacilli has long been recognized. For the purposes of this study, the term "atypical acid-fast bacillus" is a general designation for acid-fast microorganisms, isolated from human material, which vary in some respect (morphologic, pathogenetic, cultural, or biochemical) from *Mycobacterium tuberculosis* var. *hominis*. This group includes the partially acid-fast genus Nocardia as well as Mycobacterium.

Previous approaches to investigation of these microorganisms have utilized various combinations of taxonomic, cultural, biochemical, and clinical characteristics of individual strains. The literature ranges from the early studies such as those published by Thomson (1) and Pinner (2) through the more recent publications of Tarshis and Frisch (3), Buckle and Tolhurst (4), Yegian and Budd (5), Collins (6), Viallier and Bertoye (7), and Gordon and Smith (8, 9). The variety of criteria employed by the various authors has resulted in little comparative data, and the time and media requirements for their methods have not been readily adaptable to the examination of large numbers of cultures in the diagnostic laboratory.

The objective of the present study was to develop a system which is sufficiently simple in medium, technique, and time requirement for routine use, and which would yield at least group identification. The problem was approached by a comparative study of identified microorganisms to establish their cultural patterns on common laboratory media. In this report, the method used and the results obtained with 12 of the available type of cultures of acid-fast species are presented.

MATERIALS AND METHODS

The cultures tested in the study were:
Mycobacterium tuberculosis var. *hominis* H37Rv
Mycobacterium tuberculosis var. *bovis* ATCC 9834
Mycobacterium lacticola ATCC 9626
Mycobacterium fortuitum ATCC 6841
Mycobacterium avium ATCC 4676
Mycobacterium sp. ATCC 9031

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[1] From the Hektoen Institute for Medical Research of the Cook County Hospital; and the Department of Medicine, Northwestern University Medical School, Chicago, Illinois.

[2] Presented in part at the annual meeting of the Society of American Bacteriologists, New York, New York, May, 1955.

[3] Supported by grants (E 483, 483C, and 483CS) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

Mycobacterium smegmatis R 3[4]
Mycobacterium phlei R 34[4]
Nocardia madurae[4]
Nocardia braziliensis (mexicana)[4]
Nocardia asteroides[4]
Nocardia sp.[4]

The media used were:
Dehydrated beef and yeast extract broth[5]; glycerol agar slants (nutrient agar + 7 per cent glycerin); Littman oxgall agar[6]; cornmeal agar[6]; eosin methylene blue[6]; and blood (tryptose blood agar base[6] + 5 per cent blood).

All cultures were inoculated into dehydrated beef and yeast extract broth and incubated at 37°C. The time required for production of a sufficient inoculum varied from five days for the species showing surface growth to ten days for those with a bacterial type of growth. All microorganisms remained viable after thirty days in the broth when screw-capped tubes were used.

Centrifuged sediment of the growth in dehydrated beef and yeast extract broth was inoculated by sterile swab onto Littman's agar, eosin methylene blue, blood, and cornmeal agar plates, and was incubated at 37°C. The streaks were made "spoke-fashion," six to eight species to the plate. Swabs were pressed out before streaking to prevent spatter or spread of inocula, but isolated colonies are not essential. For preservation of cultures similar inoculum was placed on glycerol agar slants which supported growth within ten days at 37°C. of all species except *M. tuberculosis* var. *hominis* and *N. madurae*.

Results

The growth pattern of the cultures in the dehydrated beef and yeast extract broth divided the organisms into four distinct groups (table 1): (*1*) *M. tuberculosis* var. *hominis* and *N. madurae* failed to show visible growth in the medium in ten days although the recovered microorganisms were viable on transfer. (*2*) *M. tuberculosis* var. *bovis* and *M. lacticola* developed a bacterial type of suspension and sediment. (*3*) *M. fortuitum* and *N. braziliensis* (mexicana) formed delicate, easily broken surface growth with light frost on the tubes and large amounts of loose sediment. (*4*) *N. asteroides*, *N. sp.*, *M. avium*, *M. smegmatis*, *M. phlei*, and *M. sp.* formed tough pellicles with opaque frost on the glass and small amounts of loose sediment.

Four of the species grew on Littman's agar. *M. fortuitum* in twenty-four hours presented white, moist, and pasty growth. *M. smegmatis* and *M. phlei* grew in forty-eight hours as dry, rough pastes which were white and yellow, respectively. *M. sp.* appeared in three to four days as a dry, flat paste. All discolored the medium yellow, but this was pronounced with *M. fortuitum*.

Eight species grew well on eosin methylene blue in forty-eight hours at 37°C. producing three major patterns. *M. fortuitum* growth was moist and opaque. *M. avium* and *M. sp.* produced transparent films on the medium. *M. smegmatis*, *M. phlei*, and the three *Nocardia sp.* appeared as flat, dry, opaque growths which were indistinguishable.

[4] Strains supplied through the kindness of Dr. Ruth E. Gordon, Rutgers University, and Dr. Esther Meyer, University of Illinois.
[5] Manufactured by Difco Manufacturing Company as Bacto-Penassay-Broth.
[6] Manufactured by Difco Manufacturing Company.

TABLE 1

DEHYDRATED BEEF AND YEAST EXTRACT BROTH TWO TO TEN DAYS AT 37° C.

		Glycerol	Littman	Cornmeal	Eosin Methylene Blue	Blood
No visible growth	*M. tuberculosis* var. *hominis* H37Rv	—	—	—	—	—
	N. madurae	—	—	—	—	—
Bacterial	*M. tuberculosis* var. *bovis* ATCC 9834	white	—	—	—	—
	M. lacticola ATCC 9626	yellow	—	—	—	—
Delicate pellicle and frost	*M. fortuitum* ATCC 6841	white moist	white moist	white moist	opaque moist	white moist
	N. braziliensis (mexicana)	yellow leather	—	yellow and chalk	flat dry	depressed leather
Tough pellicle and frost	*N. sp.*	yellow leather	— —	yellow and chalk	flat dry	brown grainy
	N. asteroides	yellow leather	— —	yellow and chalk	flat dry	chalk
	M. avium ATCC 4676	white dry	—	white dry	film	gray moist
	M. sp. ATCC 9031	tan	white sparse	yellow dry	film	yellow moist
	M. smegmatis R 3	gray dry	white dry	white dry	flat dry	white dry
	M. phlei R 34	yellow dry	yellow dry	yellow dry	flat dry	yellow dry

The same eight organisms grew well on blood agar in forty-eight hours again with three major patterns. *M. fortuitum*, *M. avium*, and *M. sp.* all presented moist surfaces. *M. smegmatis* and *M. phlei* were dry, cream, and yellow, respectively, at seven to ten days. The Nocardias were flat and dry, and differentiated specifically. *N. braziliensis* (mexicana) was leathery, wrinkled, and had sharply defined depressed edges. *N. sp.* was brown, with a rough, humped surface which was grainy in texture. *N. asteroides* was distinctive in producing a profuse, chalky surface, usually in twenty-four hours.

The cornmeal agar supported growth of the same eight microorganisms which

grew on eosin methylene blue and blood agar. The surface growths offered little assistance in identification, but this medium allowed easy examination of the respective growth patterns through the bottom of the dish with low microscopic power. All of the Nocardia species presented a characteristic pattern of loose, extensive, random branching. *M. fortuitum* showed a distinctive colony edge of multiple central stems with side arborization. The other microorganisms, *M. smegmatis*, *M. phlei*, *M. avium*, and *M. sp.* presented a nondistinctive appearance of crowded, defined edges which occasionally showed a suggestion of filaments but were principally unorganized in character.

Summary

A cultural scheme, applicable for use in the routine laboratory, is presented for identification of atypical acid-fast bacilli. The "atypical" strains were separated from *Mycobacterium tuberculosis* var. *hominis* by their ability to grow in dehydrated beef and yeast extract broth (Penassay®) at 37°C. and subsequently on glycerol agar in less than ten days.

The genus Nocardia was identified by its failure to grow on Littman's agar and by its colony characteristics on other media. *Nocardia madurae* failed to grow at 37°C.; the other three species of *Nocardia* were differentiated from each other on blood media.

Mycobacterium fortuitum was recognized by its distinctive moist and rapid growth on all media. The other six organisms fell into pairs: *Mycobacterium avium* and *Mycobacterium sp.* were separable by use of Littman's medium; *Mycobacterium smegmatis* and *Mycobacterium phlei* were separable by color; and *Mycobacterium bovis* and *Mycobacterium lacticola* were distinguished by bacterial-like growth in broth and were separable from each other by color.

Sumario

Bacilos Acidorresistentes Atípicos. I. Plan Cultural para Identificación Rápida

Ofrécese un plan cultural, aplicable para empleo en la mayoría de los laboratorios, para identificación de los bacilos ácidorresistentes atípicos. Se separaron las cepas "atípicas" de las del *Mycobacterium tuberculosis* var *hominis* por su capacidad para proliferar en el caldo de Penassay a 37° C. y después en agar-glicerol en menos de diez días.

El género *Nocardia* se identificó por su falta de proliferación en el agar de Littman y por las características de sus colonias en otros medios. La *Nocardia madurae* no proliferó a 37° C; las otras tres especies de *Nocardia* fueron diferenciadas entre sí en medios de sangre.

El *Mycobacterium fortuitum* fué reconocido por sus distintivas y rápidas colonias húmedas en todos los medios. Los otros seis microbios se separaron en pares: El *Mycobacterium avium* y el *Mycobacterium* sp. resultaron separables con el uso del medio de Littman; el *Mycobacterium smegmatis* y el *Mycobacterium phlei* fueron separables por su color; y el *Mycobacterium bovis* y el *Mycobacterium lacticola* se distinguieron por sus colonias bacteriformes en caldo, siendo mutuamente separables por el color.

Resume

Bacilles acido-résistants atypiques: I. Un schéma cultural pour l'identification rapide

Présentation d'un procédé de culture applicable dans un laboratoire ordinaire pour l'identification des bacilles acido-résistants. Les souches "atypiques" sont séparées du

Mycobactérium tuberculosis var. *hominis* par leur capacité de pousser sur le bouillon Penassay à 37°C et ensuite dans le milieu glycérol-gélose en moins de dix jours.

Le genre *Nocardia* est identifié par le défaut de croissance sur le milieu Littman-gélose et par les caractères de ses colonies dans les autres milieux. Le *Nocardia madurae* ne pousse pas à 37°C; les trois autres espèces de *Nocardia* se différencient l'une de l'autre sur les milieux au sang.

Le *Mycobactérium fortuitum* est identifié par le caractère distinctif de sa croissance humide et rapide sur tous les milieux. Les six autres microorganismes sont isolés en paires: le *M. avium* et le *Mycobactérium sp.* sont différenciés à l'aide du milieu de Littman; le *Mycobactérium smegmatis* et le *Mycobactérium phlei* sont différenciés par coloration; le *Mycobactérium bovis* et le *Mycobactérium lacticola* se distinguent par leur croissance dans le boullion qui est analogue à celle des bactéries, ils sont différenciés l'un de l'autre par coloration.

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ATYPICAL ACID-FAST BACILLI[1,2,3,4]

II. Mycobacterium Fortuitum: Bacteriologic Characteristics and Pathogenicity for Laboratory Animals

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INTRODUCTION

Among the heterogeneous atypical acid-fast organisms, *Mycobacterium fortuitum* is one of the few taxonomically distinct species (1) characterized by rapid growth and nonfastidious cultural requirements. Rough fortuitum colonies may grossly resemble those of *Mycobacterium tuberculosis* var. *hominis* on initial isolation, but growth appears as early as three to five days after inoculation.

The frequency of the occurrence of *M. fortuitum* among several hundred atypical mycobacteria isolated from patients, coupled with clinical observations presented by Cruz (2) and Wells and associates (3), invited further study of this mycobacterial species. A comparative investigation of 28 strains was undertaken to validate simple cultural differentiation, to further characterize the species, and to study experimental infection.

OBSERVATIONS

Bacteriologic Studies

Cultural behavior: To control this study, 11 stock cultures of *Mycobacterium fortuitum* were obtained (table 1). To these were added 17 strains newly isolated from patients at Cook County Hospital (table 2).

The identification of these microorganisms was made by a system previously described (4), using simple bacteriologic diagnostic media (table 3). Identification of six new isolates was confirmed taxonomically by Dr. Ruth E. Gordon. Using this method, 27 of the 28 strains gave minimal variations on all media. The one exception was a stock strain (No. 48), originally isolated from soil, which grew too slowly for the purpose of this study.

Variation among strains was limited to slight differences in growth rates and moisture of colonies. The greatest variability appeared in the morphology of the organisms which ranged from coccobacillary to almost filamentous in length (figure 1). The species showed a tendency to produce granular bodies which were broader than the cell diameter and which stained more intensely. Individual

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[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 21, 1956.

[3] Presented in part at the annual meeting of the Society of American Bacteriologists, New York, New York, May, 1955.

[4] Supported by grant RG-4523(C2) from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

TABLE 1

STOCK CULTURES OF M. FORTUITUM

Laboratory Number	Original Identification	Source
ATCC 6841	*Mycobacterium fortuitum* Cruz (J. C. Cruz, Instituto Oswaldo Cruz, Brazil)	Human abscess
NCTC 8697	*Mycobacterium sp.*	Human abscess
480	*Mycobacterium minetti* Penso *et al.* (G. Penso, Istituto Superiore de Sanita, Rome)	Cow (C70 of Minett)
NCTC 6926	*Mycobacterium enteritidis* Lehmann and Neumann	
NCTC 8573	*Mycobacterium sp.* (E. Aguis, Malta)	Cervical adenitis
394	*Mycobacterium sp.* (W. A. Hagan, Cornell University)	Bovine mastitis
456	*Mycobacterium sp.* (E. W. Tucker, Cornell University)	Bovine mastitis
NCTC 2291	*Mycobacterium sp.*	Halibut
48	*Mycobacterium sp.* (W. A. Hagan, Cornell University)	Soil
389	*Mycobacterium sp.* (G. Alexander, Rutgers University)	Barnyard soil
	Mycobacterium Aguis (E. H. Runyon)	

TABLE 2

PATIENT STRAINS OF M. FORTUITUM

Source	Number	Fate of Patient	Number	Tubercle Bacilli Isolated Concurrently
Sputum	9	Dead*	5	0
		Alive	4	0
Bronchoscopy	3	Dead	1	0
		Alive	2	1
Scalene node	3	Dead	1	1
		Alive	2	1
Skin ulcer	1	Alive	1	0
Liver—PM	1	Dead	1	1

* Patients died before isolation and identification of organism was completed. Clinical diagnosis was tuberculosis.

TABLE 3

SCHEME OF CULTURAL IDENTIFICATION

Medium	Time	Character	Acid-fast
	days		
(1) Isolation for tubercle bacilli	3–12	Rough or smooth soft texture, white to cream	+
(2) Dehydrated beef and yeast extract broth	1–4	Surface growth easily dislodged, light frost, fluid clear	+
(3) Littman plate	2	White, soft moist surface, discolors medium yellow	+
(3) Eosin methylene blue plate	2	Opaque growth, moist surface	±
(3) Blood agar base + 5 per cent whole blood, plate	2	Cream, soft moist surface	±
(3) Cornmeal plate	2	Thin growth, white, branching edge	+

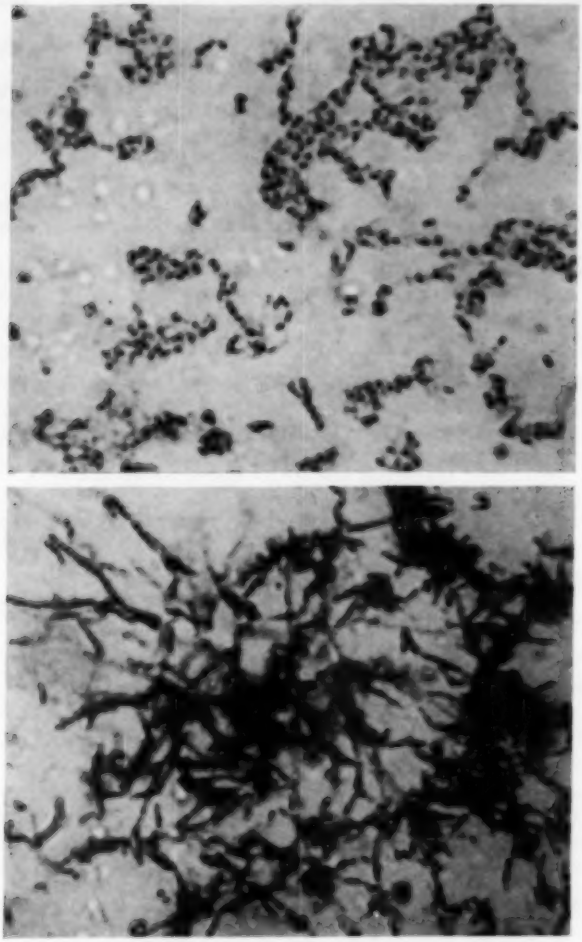

FIG. 1. *M. fortuitum*: Ziehl-Neelsen stain of smears from selected cultures showing extremes of morphologic variants: (Upper) Cocobacillary rods. (× 980). (Lower) Filamentous rods containing multiple granules. (× 980)

strains were themselves variable, however, both in length and granule formation. No correlation was found between morphologic variants and particular strains, media, or age of cultures.

The species is strongly acid fast, but on certain media (eosin methylene blue and those containing blood or glycerol) more than 50 per cent of the cells resisted both fuchsin and the counterstain. This reaction occurred with all of the morphologic variants.

TABLE 4

DRUG SUSCEPTIBILITY OF M. FORTUITUM IN VITRO

Drug	Concentration	Strains Tested	Inhibition
	γ		
3,4-Dimethyl-5-sulfanilamido isoxazole	50.0	19	
Penicillin G	10.0	19	
Bacitracin	20.0	19	
Neomycin	30.0	19	
Polymyxin	30.0	19	
Erythromycin	10.0	19	
Chloramphenicol	50.0	19	
Oxytetracycline	60.0	19	8
	10.0	19	8
Hydroxystilbamidine	100.0	6	
Propamidine	100.0	6	
Acidione	100.0	6	
Streptomycin	100.0	27	
PAS	25.0	27	
Isoniazid*	0.5	27	
	1.0	27	27
Tetracycline*	1.0	27	
	5.0	27	27

* Tube dilution method

Resistance and viability: M. fortuitum is fully as resistant a species as is *M. tuberculosis*. Pure cultures survived sodium hydroxide digestion at 37°C. for one hour. Cultures freshly inoculated into dehydrated beef and yeast extract broth[5] and heated at 80°C. for ten minutes produced, after ten days of incubation at 37°C., only scant sedimented growth, but resumed normal rate of surface growth when transferred to fresh broth. Broth cultures evaporated to dryness and held at 37°C. for five days also yielded viable organisms with typical growth upon transfer. Cultures have remained viable for as long as one year in sealed tubes of Sabouraud and glycerol agar. There was no variation in survival among the 27 strains.

Drug susceptibility in vitro: Inhibition tests were performed by the disc method to determine the *in vitro* activity of 15 bacteriostatic agents. The medium used was dehydrated beef and yeast extract agar and the growth was recorded at forty-eight hours (table 4). In the concentrations used, only one antimicrobial agent, oxytetracycline, was inhibitory, and the drug showed this property in less than 50 per cent of the strains tested. Of the four known drugs to inhibit the *in vitro* growth of *M. tuberculosis*, isoniazid and tetracycline were inhibitory to all strains of *M. fortuitum* at concentrations practically attainable *in vivo*, whereas streptomycin and PAS showed no activity even at high concentrations.

[5] Manufactured as Bacto-Penassay-Broth by Difco Manufacturing Company.

TABLE 5

TIME OF APPEARANCE OF LESIONS (M. FORTUITUM)
Intraperitoneal Inoculation—12 Strains

	Number of Animals	Time	Lesions with Acid-fast Bacilli	Per Cent
		days		
Died	9	12 (6–28)	9	100
Sacrificed	14	21	8	57
	13	28	11	85
	34	35	20	59
Total	70		48	69

Experimental Infection of Mice with M. Fortuitum

Virulence: Twelve strains, 8 new isolates, and 4 stock strains of *M. fortuitum* were selected for pathogenicity studies. Seventy-two male Swiss white mice (averaging 20 gm.) were inoculated intraperitoneally in groups of 6 with each strain. A noninoculated group of 6 animals served as controls. The inoculum was the centrifuged sediment of four-day-old dehydrated beef and yeast extract broth cultures suspended in sterile 0.9 per cent saline solution. The density of inoculum was approximated to the turbidity of the McFarland Nephelometer Standard No. 5.[6] Each mouse received 0.5 ml. of the prepared inoculum.

Evaluation of animals for the presence of lesions consisted of examination of gross lesions by Ziehl-Neelsen stain of direct smears and histologic preparations of formalin fixed tissue; and routine histologic sections from liver, spleen, kidney, and lung. All tissue sections were stained with hematoxylin and eosin and Ziehl-Neelsen by the Kinyoun cold technique. Lesions were accepted as specific only upon demonstration of acid-fast bacilli either in direct smears or in histologic preparations.

Seventy mice survived inoculation (table 5). Nine died with specific lesions between the sixth and twenty-eighth days. The survivors (87 per cent of the total) were sacrificed in three groups, at twenty-one, twenty-eight, and thirty-five days after inoculation. It is apparent that the greatest incidence of infected animals (85 per cent) occurred at four weeks. The over-all incidence of positively infected animals was lower (69 per cent) by virtue of the smaller number of lesions found at three and five weeks.

The organs most frequently involved were liver and spleen (table 6), with a diminishing frequency of involvement of kidney, lung, and intraperitoneal abscesses. The distribution of lesions was roughly comparable in the animals with progressive fatal disease and in those sacrificed at four weeks.

Comparison of virulence (table 7) revealed no significant differences among the 12 strains studied. All strains infected at least 50 per cent of the animals, and 8 (including 3 of the stock cultures) produced progressive fatal disease.

Macroscopic pathologic findings: The liver and spleen were most frequently involved, showing white to cream-colored capsular and subcapsular abscesses

[6] Plate counts performed on three suspensions prepared and standardized by the described method yielded counts of 12×10^9, 14×10^9, and 15×10^9 viable organisms per ml., respectively.

TABLE 6

DISTRIBUTION OF LESIONS IN MOUSE TISSUE

Intraperitoneal Inoculation

	Dead	Sacrificed	Total
Fate	9 (13%)	61 (87%)	70 (100%)
Total mice with acid-fast bacilli	9 (100%)	39 (64%)	48 (69%)
Liver	6	31	37 (53%)
Spleen	6	19	25 (36%)
Kidney	4	9	13 (19%)
Lung	4	0	4 (6%)
Miscellaneous abscess	3	5	8 (11%)

TABLE 7

VIRULENCE OF M. FORTUITUM ACCORDING TO INDIVIDUAL STRAINS*

Number of Strains	Number of Animals	Number of Infected	Number of Deaths	Rate of Infection
				per cent
6	36	24	6	66
3	16	8	1	50
2	12	10	2	83
1	6	6	0	100

* Strains included stock strains NCTC 6841, NCTC 8573, 480, *M.* Aguis, and 8 new isolates, inoculated intraperitoneally.

ranging in size from 1 to 8 mm. The abscess contents were usually liquefied or purulent and, only occasionally, frankly caseous. Frequently an abscess in the left lobe of the liver was adherent to the spleen (figure 2). The spleen was slightly to moderately enlarged. The kidneys showed frequently unilateral, small subcapsular abscesses which only occasionally extended deep into the parenchyma. The lungs showed only focal areas of congestion and atelectasis; abscesses were rarely encountered. Intra-abdominal lymph nodes were enlarged and adherent to the under surface of the liver, the spleen, pancreas, stomach, and abdominal wall. Necrotic nodules and abscesses occasionally united the dome of the liver to the diaphragm.

Histologic observations: Evaluation of microscopic tissue lesions increased by 25 per cent the over-all incidence of lesions, and also served to confirm the specificity of occasional gross lesions in which acid-fast bacilli could not be demonstrated on direct smear.

Specific tissue alterations (associated with the presence of acid-fast bacilli) occurred in liver, spleen, kidney, lung, lymph nodes, diaphragm, and, rarely, in the pancreas. The lesions were most commonly abscesses of similar appearance in all organs; frankly granulomatous lesions occurred infrequently.

Capsular involvement was frequent in liver (figure 3), spleen (figure 4), and kidney (figure 5), and consisted of fibrinous exudate and diffuse but mild cellular infiltration by histiocytes, segmented leukocytes, lymphocytes, and occasional

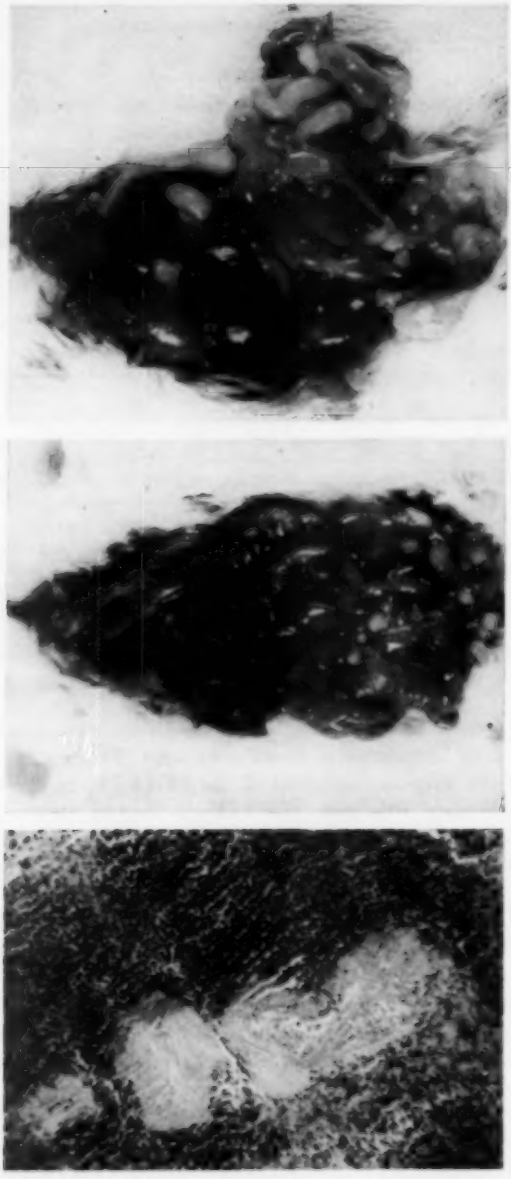

FIG. 2. (Upper) Subcapsular abscesses in liver, spleen, and kidney. Mouse sacrificed on the twenty-eighth day after intraperitoneal inoculation with *M. fortuitum*. (macroscopic). (Center) Generalized lymphadenitis in mouse sacrificed on the twenty-eighth day after intravenous inoculation. (macroscopic). (Lower) Mesenteric lymph node abscess with central necrosis, six days after intraperitoneal inoculation. (× 100)

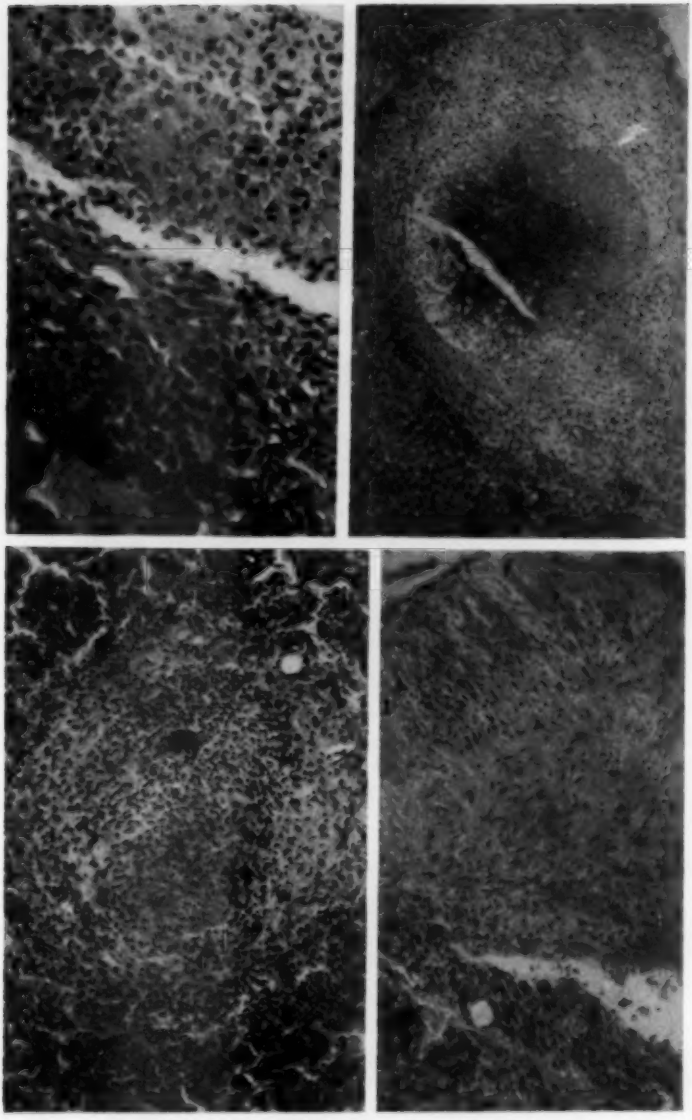

FIG. 3. Stages of hepatic lesions (*M. fortuitum* inoculated intraperitoneally): (Upper left) Capsular granuloma at twenty-first day. (× 240). (Upper right) Subcapsular abscess with necrosis at twenty-eighth day. (× 65). (Lower left) Granuloma with epithelioid cells and histiocytes predominating at twenty-eighth day. (× 120). (Lower right) Healing granuloma at twenty-first day. (× 110)

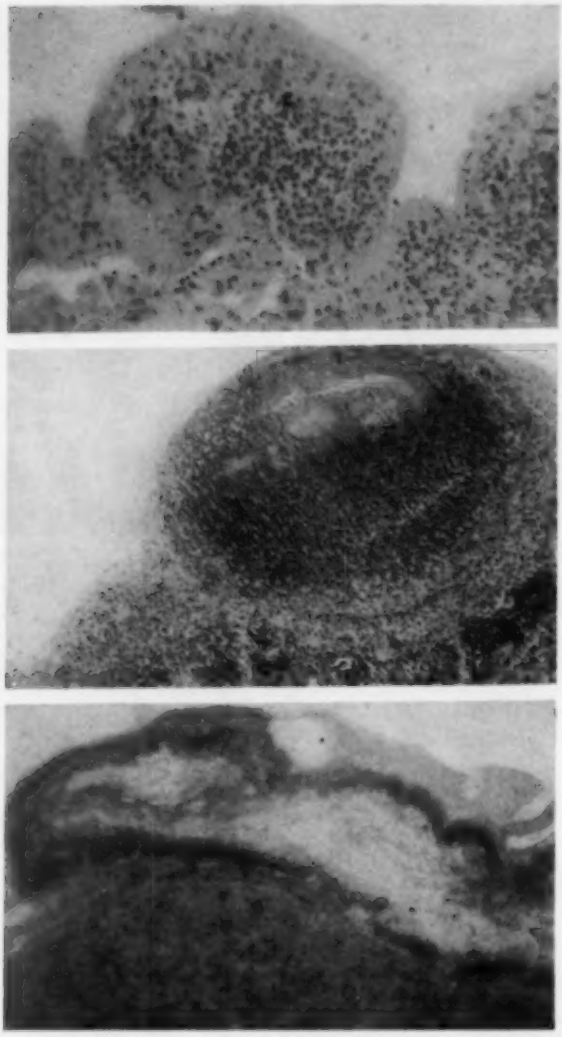

FIG. 4. Splenic lesions (six days after intraperitoneal inoculation of *M. fortuitum*): (Upper) Early capsular granuloma (× 120). (Center) Subcapsular abscess with early central necrosis. (× 65). (Lower) Extensive capsular caseation necrosis. (× 65)

plasma cells. In some segmented leukocytes, in others histiocytes, predominated. The capsule was often diffusedly thickened, showing nodular cellular accumulations and occasionally microabscesses with central necrosis. Diffuse necrosis with caseation occurred rarely.

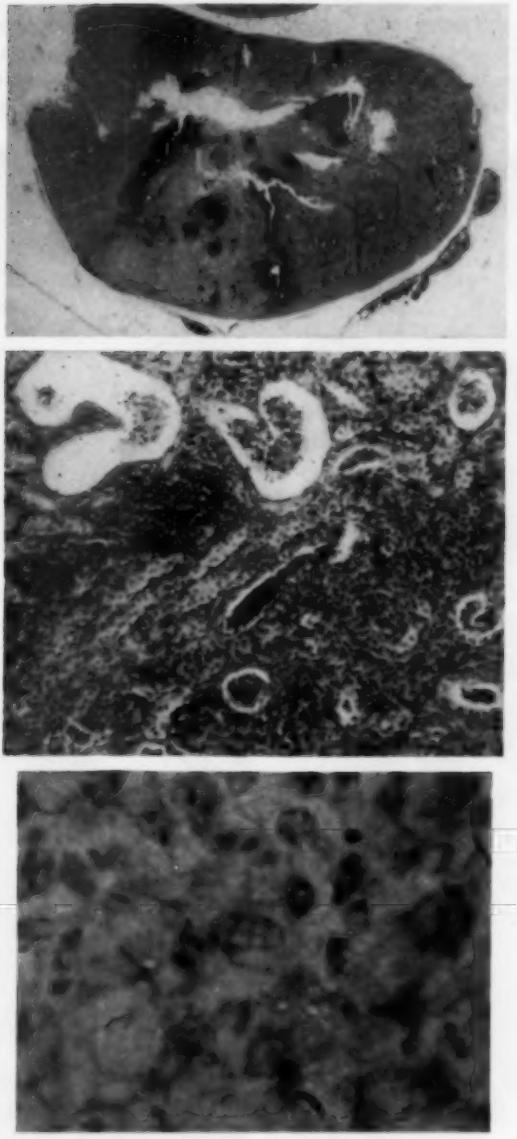

FIG. 5. Renal lesions: (Upper) Extensive cortical necrosis, seventh day, in mouse inoculated intraperitoneally. (× 6). (Center) Severe interstitial nephritis in mouse inoculated intravenously, sacrificed at 56 days. (× 65). (Lower) Acid-fast bacilli in interstitial nephritis. (× 980)

Subcapsular abscesses, most frequent in liver, spleen, and kidney, were limited by a pyogenic membrane composed of vascular connective tissue and segmented leukocytes. The necrotic and frequently hyalinized center contained cellular debris, nuclear fragments and fibrin, surrounded by a zone of segmented leuko-

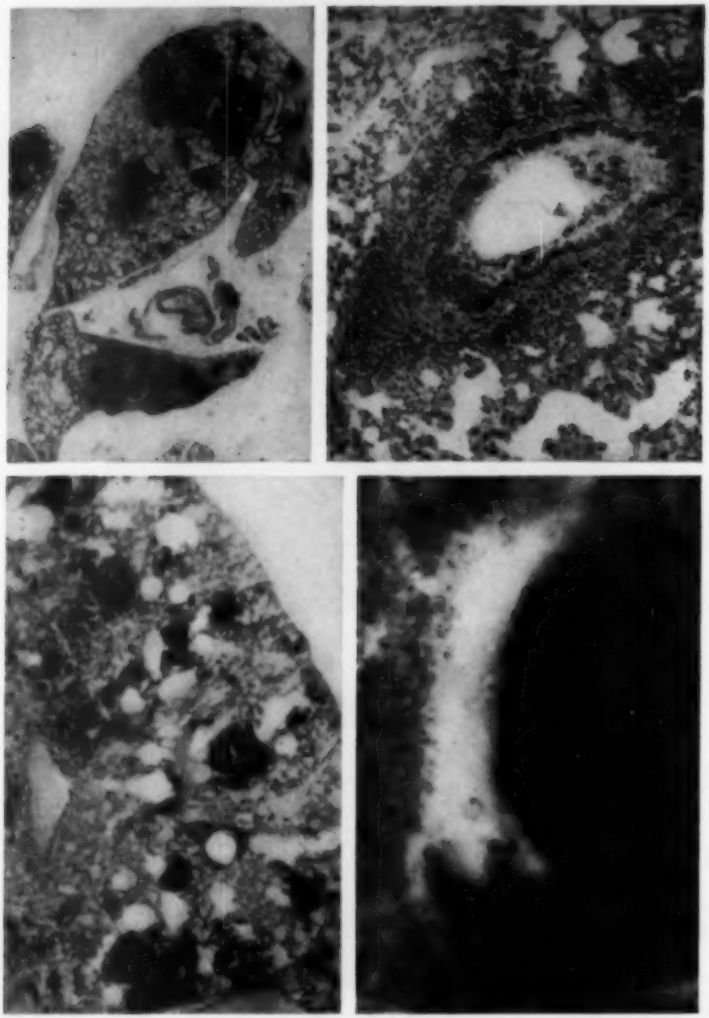

FIG. 6. Pulmonary lesions: (Upper left) Multiple abscesses, seventh day, in mouse inoculated intraperitoneally. (× 6). (Upper right) Nonspecific peribronchitis and interstitial pneumonitis, mouse sacrificed 21 days after intraperitoneal inoculation. (× 65). (Lower left) Multiple necrotic nodules, third day, in mouse inoculated intravenously. (× 6). (Lower right) Masses of acid-fast bacilli in necrotic pulmonary nodule. (× 900)

cytes, histiocytes, lymphocytes, fibroblasts, and sometimes epithelioid cells. Such lesions less frequently were composed largely of mononuclear cells and resembled small tubercle-like structures. Likewise, pulmonary (figure 6) and lymph node abscesses often showed, at their periphery, well-circumscribed nodules of histiocytes, lymphocytes, and epithelioid cells.

Discrete granulomas were rarely encountered, consisting of central fibrinous exudate and predominance of histiocytic cells, occasionally multinucleated, with foamy or eosinophilic cytoplasm; segmented leukocytes were found in the periphery where they accompanied newly formed capillaries, lymphocytes, and fibroblasts. In healing phases epithelioid cells as well as histiocytes were prominent.

Three other types of lesion were occasionally observed: extensive renal cortical necrosis, severe interstitial nephritis and pyelonephritis with interstitial necrotic nodules and vascular necroses, and infarction due to vascular thrombosis by exudate containing numerous acid-fast bacilli.

Nonspecific alterations (not associated with acid-fast bacilli) included severe passive congestion, focal collections of histiocytes, lymphocytes, and segmented leukocytes, areas of focal necrosis in the liver, diffuse reactive hepatitis, presence of megakaryocytes in the liver and more often in the spleen, acute splenitis and hyperplasia, mild pyelonephritis, pleurisy, peribronchitis, and interstitial pneumonia, and occasional cutaneous abscesses.

Infection by the intravenous route: The preceding experiments were duplicated, except that the dose of inoculum was 0.1 ml. injected into the tail vein. Twenty-six per cent of the animals died, two-thirds within fifteen days, and of these half had specific lesions. The remainder were sacrificed at fifty-six days. The over-all incidence of infected animals was low, only 27 per cent of the total (table 8).

A comparison of these animals with the intraperitoneally inoculated mice revealed a striking difference in the distribution of lesions, with intravenous infection resulting in predominant renal and pulmonary involvement and generalized lymphadenitis (figure 2). Specific lesions were uniformly abscesses of the same character as those which occurred in the intraperitoneal group. Necrotic pulmonary nodules contained large masses of acid-fast bacilli (figure 6), but no granulomas were observed. The nonspecific lesions were identical.

In extension of the comparison between the two routes of inoculation, a study was made of 6 additional strains of *M. fortuitum*, inoculated according to the

TABLE 8

DISTRIBUTION OF LESIONS IN MOUSE TISSUE

Intravenous Inoculation

	Dead	Sacrificed	Total
Fate	18 (26%)	52 (74%)	70 (100%)
Total mice with acid-fast bacilli	9 (50%)	10 (20%)	19 (27%)
Kidney	7	9	16 (23%)
Lung	4		4 (6%)
Liver	1		1 (1%)
Spleen		1	1 (1%)

TABLE 9

DISTRIBUTION OF LESIONS IN MOUSE TISSUE

Six Stock Strains*

	Sacrificed
Intraperitoneal Inoculation	
Fate	34
Total mice with acid-fast bacilli	29 (85%)
Kidney	11 (32%)
Liver	15 (44%)
Spleen	7 (20%)
Other	11 (32%)
Intravenous Inoculation	
Fate	34
Total mice with acid-fast bacilli	9 (27%)
Kidney	9 (27%)
Lymphadenitis	7 (20%)

* Strains included NCTC 8697, 394, NCTC 6929, R 457, R 389, NCTC 2291.

previous procedure both intraperitoneally and intravenously (table 9). All of the animals were sacrificed at twenty-eight days. Lesions (confirmed by direct smear only) showed a distribution characteristic for each group and the results were consistent with previous observations.

Comparison with M. tuberculosis: Six mice were inoculated intraperitoneally with *M. tuberculosis* H37Rv. All were sacrificed at four weeks. The lesions in 5 of the mice were indistinguishable in appearance and distribution from those produced by *M. fortuitum*.

Experimental Infection of the Rabbit and the Guinea Pig with M. Fortuitum

No visceral lesions were encountered following either intravenous or subcutaneous inoculation of *M. fortuitum* into rabbits. However, subcutaneous inoculation of 9 rabbits, each with a different strain, uniformly produced local abscesses and caseation, with exudate which contained numerous acid-fast bacilli.

Twelve strains of *M. fortuitum* were inoculated intraperitoneally into 12 guinea pigs. No visceral lesions were found at sacrifice at six weeks, but 5 of the animals had small abscesses at the site of inoculation with acid-fast microorganisms demonstrable by Ziehl-Neelsen stain.

Hypersensitivity: M. fortuitum antigen was prepared from a sterile broth filtrate of cultures of 9 individual strains. Both individual and pooled antigens were employed for intradermal tests.

Prior to intravenous inoculation, skin tests in 15 rabbits were negative with human, avian, bovine, and mixed *M. fortuitum* tuberculin material. At ten to twelve weeks after intravenous inoculation of *M. fortuitum* cultures, skin tests in the sensitized rabbits showed no reaction with human tuberculin but were uni-

formly positive with avian, bovine, and *M. fortuitum* antigens (both individual and pooled), the fortuitum producing the most marked reaction.

Discussion

M. fortuitum has emerged as a well-defined species from a large heterogeneous group of atypical mycobacteria.

The 27 strains included in the present study show no significant bacteriologic variations. Discrepancies with the results of others (1–3, 5) are attributable to differences in techniques. The fact that identification of some of the strains was arrived at by two independent methods, rapid cultural identification (4) and taxonomic study (6), argues for the homogeneity of the species. The agreement also appears to validate rapid cultural identification as a method.

The uniform resistance of *M. fortuitum* to streptomycin and PAS *in vitro* and its susceptibility to isoniazid conform with the reactions of other atypical mycobacteria (7). *M. fortuitum* shares susceptibility to tetracycline with tubercle bacillus of human origin (8); this property may be of some clinical significance.

M. fortuitum is highly pathogenic in mice. It is not necessary to enhance the experimental infection by the use of Tween®-albumin medium, mucin suspension of inoculum, or the selection of a highly susceptible host, such as a pigmented strain of mice. However, a heavy inoculum, in the order of 14×10^9 organisms per ml. was used. The distribution and character of the lesions is similar to those observed by Wells (2). The distribution appears to be determined by the route of inoculation rather than by organ specificity.

As in mouse tuberculosis, the tissue response to *M. fortuitum* resembles a bacteremic infection. Most of the lesions are abscesses, but many have granulomatous characteristics, and some discrete granulomas occur. The character and distribution of lesions are not essentially different from those caused by *M. tuberculosis*.

The differentiation of *M. fortuitum* from *M. tuberculosis* could be made on animal inoculation using both mice and guinea pigs. Both are pathogenic in mice but *M. fortuitum* fails to infect the guinea pig. The more practical and definitive identification of *M. fortuitum* rests on its microbiologic properties of extremely rapid growth on ordinary media, surface growth in broth, and resistance to streptomycin and PAS.

Summary

Comparative bacteriologic studies of seventeen new isolates of *Mycobacterium fortuitum* and 10 previously identified strains showed complete uniformity. The species was further characterized by *in vitro* resistance to streptomycin and PAS, and susceptibility to isoniazid and tetracycline. *Mycobacterium fortuitum* cross-reacted with avian and bovine but not with human tuberculin.

All strains were pathogenic in mice, producing a disseminated infection associated with lesions intermediate between bacterial abscesses and granulomas. Rabbits and guinea pigs could not be infected.

Sumario

Bacilos Acidorresistentes Atípicos. II. Mycobacterium Fortuitum. Características Bacteriológicas y Patogenicidad para los Animales de Laboratorio

Estudios bacteriológicos comparados de diecisiete nuevas cepas aisladas del *Mycobacterium fortuitum* y de diez cepas identificadas anteriormente revelaron absoluta uniformidad. La especie se caracterizó además por resistencia *in vitro* a la estreptomicina y al PAS y sensibilidad a la isoniacida y la tetraciclina. El *Mycobacterium fortuitum* mostró reacciones cruzadas con la tuberculina aviaria y la bovina, pero no con la humana.

Todas las cepas resultaron patógenas en los ratones, produciendo septicemia asociada con lesiones intermedias entre abscesos y granulomas bacterianos. No se pudo infectar a conejos y cobayos.

Resume

Bacilles atypiques acido-résistants: II. Mycobactérium fortuitum. Caractères bactériologiques et pathogénicité chez l'animal expérimental

Des recherches bactériologiques comparatives sur dix-sept nouveaux isolats de *Mycobactérium fortuitum* et dix souches antérieurement identifiées ont démontré leur uniformité complète. Les espèces étaient en outre caractérisées par une résistance *in vitro* à la streptomycine et au PAS et une sensibilité à l'isoniazide et à la tétracycline. Le *Mycobactérium fortuitum* donnait une réaction croisée avec la tuberculine aviaire et bovine, mais non avec la tuberculine humaine.

Toutes les souches étaient pathogènes pour la souris et produisaient une septicémie à laquelle s'associaient des lésions intermédiaires entre l'abcès bactérien et le granulome. Les lapins et les cobayes ne pouvaient pas être infectés.

Acknowledgment

The writers are indebted to Dr. Ruth E. Gordon for her confirmatory studies of six new isolates and for her cooperation in providing selected stock strains of *Mycobacterium fortuitum* for comparative study.

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Case Reports

CORTISONE THERAPY IN HAMMAN-RICH SYNDROME[1]

Report of a Case

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(Received for publication October 8, 1956)

Introduction

Accumulating clinical reports indicate that acute diffuse interstitial fibrosis, or the Hammon-Rich syndrome, not only is more common, but also may be less acute and rapidly progressive, than was originally described (1–3). The advent of steroid therapy of the pituitary-adrenocortical type in turn has centered interest on the possibility of reversing or arresting the prefibrotic changes (4, 5). The dangers of such therapy, particularly abrupt discontinuance, have been reported (6, 7).

The present case is reported because it was among the first to be diagnosed by histologic study during the life of the patient (8, 9), and was subjected to prolonged steroid therapy with some measure of clinical improvement; the date of roentgenographically demonstrable onset could be set with fair accuracy; the patient survived three years and five months from the establishment of the diagnosis; and, finally, necropsy data for comparison with the original biopsy are available.

Case Report

The patient was a white, 61-year-old female, who was admitted to the Cedars of Lebanon Hospital on July 1, 1951. The current and past family history was not pertinent to the present illness. The patient had suffered with headaches diagnosed as migraine for ten years. One year previously, she was said to have had osteoarthritis which accounted for pains in the back and arms. A roentgenogram of the chest at that time showed essentially normal heart and lungs, except that the lungs appeared moderately overaerated.

For the past two years the patient had had a moderate cough with expectoration of mucoid sputum. This had become worse in the last six months. A weight loss of 20 pounds had occurred in the past year, and for six months the patient had noted swelling of the terminal digital phalanges. At times this was moderately painful, and the pain was increasing in severity. In recent months, there had been increasing dyspnea, especially noted on changing position, with episodes of weakness on becoming erect, which almost entirely incapacitated her by the time of her admission to the hospital.

Physical examination disclosed a normally developed and fairly well-nourished white female, lying in bed, without obvious distress, but moderately tachypneic and dyspneic on minimal exertion. There was an absence of cyanosis and a moderate clubbing of the terminal phalanges with reddened, slightly tender swelling around the nail beds (figure 1). The chest showed diminished expansion, moderate hyper-resonance, and inspiratory and expiratory sibilant rales. The heart was not enlarged; the second pulmonic sound was accentuated. Abdominal examination was negative.

A roentgenogram of the chest (figure 2*a*) showed a uniformly distributed lesion throughout

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FIG. 1. Illustration showing clubbing of fingers. Note prominence of swelling around the nail beds which was painful and part of the presenting complaint.

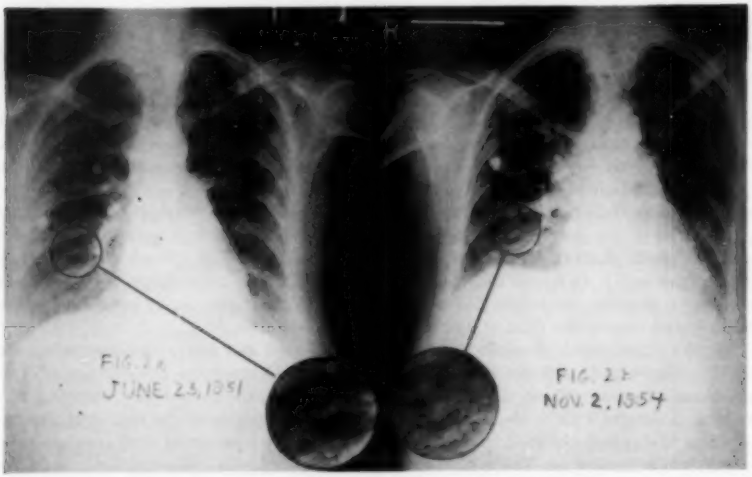

FIG. 2*a* (Left). Roentgenogram of the chest (June 23, 1951) showing a uniformly distributed lesion throughout both lung fields. Circular inserts show magnified detail.

FIG. 2*b* (Right). Roentgenogram of the chest, obtained on November 2, 1954, showing no change in lesion.

both lung fields. This consisted of a predominantly nodular pattern, some linear elements having a bronchovascular distribution. Some confluence was noted, but generally the nodules were discrete and gave the lungs a fine to coarsely granular appearance.

Laboratory data were as follows: The erythrocyte count was 4,800,000 per cu. mm.; the leukocyte count, 6,700 per cu. mm., with polymorphonuclears, 68 per cent; lymphocytes, 31 per cent; monocytes, 1 per cent. Bone marrow puncture was within normal limits and showed no evidence of granulomatous or neoplastic disease. The erythrocyte sedimentation rate was 46 mm. per hour (Cutler). Serologic tests for syphilis (Kline and Wasserman) were negative. Skin tests for coccidioidin, 1:100, were negative; the histoplasmin test, 1:1,000 dilution, was negative; first-strength PPD was negative; second-strength PPD, 1-plus. Total serum proteins were 7.7 gm. per 100 ml.; albumin, 4.2 gm. per 100 ml., and globulin, 3.5 gm. per 100 ml. Routine urine and Addis counts were within the limits of normal. Biopsy of the deltoid muscle showed periangiitis of insufficient degree of specificity to be diagnostic. Six specimens of sputum were examined for abnormal cytology and no evidence of malignancy was encountered. Sputum obtained by expectoration as well as by bronchoscopic aspiration was negative for tubercle bacilli in a number of concentrates and cultures. One culture showed a single colony of a nonpathogenic chromogenic acid-fast bacillus. Smears and stains for fat droplets were negative, as were smears and cultures for fungi. Stools were negative for blood, ova, and parasites.

Bronchoscopy, except for a slight increase in mucoid secretions, was negative. An electrocardiogram showed a right ventricular strain pattern. The Thorn test indicated 176 eosinophils per cu. mm.; after 25 units of corticotropin, 44 eosinophils per cu. mm.

On July 17, 1951, surgical exploration of the left chest was undertaken. Incision was made in the fourth anterior interspace on the left side. On opening the thorax, the pleura was free except for a few filmy adhesions. The lung was studded with palpable, small, 3 to 5 mm. nodules which had a rubbery consistency. The lymph nodes in the hilum were small and anthracotic. The biopsy was made by amputating approximately 2 inches of the lingula of the left upper lobe, using a clamp and suture method. A tube was placed for drainage. The postoperative course was uneventful.

The biopsy (figure 3) showed a portion of lung with a slightly thickened pleura. The pulmonary parenchyma was markedly altered by interstitial fibrosis. Thickening of the

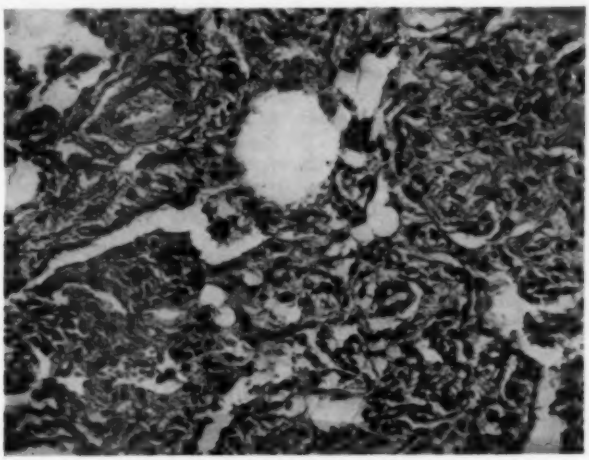

FIG. 3. Surgical specimen of lingula, left upper lobe.

alveolar walls and proliferation extending from the bronchovascular sheaths were observed. In places there was loose myxoid young connective tissue and, in others, denser collagenized stroma. Special stains revealed little change in elastica but considerable proliferation of reticulum. There were scattered nodules of lymphocytes, some of which formed herniations into the alveolar spaces. The alveolar linings were metaplastic. In places they formed intra-alveolar sheets. Atypical and giant elements and squamatoid nests were noted. There was moderate vascular sclerosis but no intrinsic angiitis. Acid-fast and fat stains were negative. The lesion was considered to be a fibrosing interstitial pneumonitis of the type described by Rich. The immature mesenchymal proliferation suggested that hormone therapy might abort its development into mature connective tissue.

The patient was given cortisone with a gratifying initial response. She received at first 200 mg. daily, beginning two days after surgery. There was a rapid subjective response in that the cough subsided almost completely, dyspnea was sharply improved, and there was a striking increase in the patient's vitality, obviously aided by a definite and pleasurable euphoria. These changes made it possible for her to be ambulant by July 26 and discharged from the hospital on July 29, 1951.

She returned to her home in a nearby community and in a month was performing all but her heavy household duties. After the first week, the cortisone was reduced to 100 mg. in twenty-four hours and, with temporary changes only, she was kept on this dosage until December, 1953.

At this point, the patient refused further medical care and discontinued the cortisone. Her symptoms had been controlled with decreasing effectiveness during 1953. She felt ill, complained of weakness and dyspnea, but was able to perform part of her household duties until May, 1954. At this time, she was admitted to the City of Hope Medical Center for further evaluation and therapy.

Routine clinical and laboratory examinations, which included blood counts, electrolyte studies, chest roentgenograms, and electrocardiograms, disclosed no distinctive changes from previous studies. The outstanding features were dyspnea, clubbed fingers, and the unchanging roentgenographic picture. During this stay, which terminated on June 18, 1954, she received cortisone, 100 mg. daily, and thyroid (desiccated), 0.5 gr. daily. There was no apparent response to these drugs.

Following discharge, the patient returned to her home and received no other treatment until further deterioration of her status necessitated her readmission to the City of Hope Medical Center on November 2, 1954.

Except for increased weakness and a further weight loss of 15 pounds, there was no specific change in her condition. The roentgenogram was unchanged (figure 2*b*), and electrolyte studies (sodium, potassium, chlorides) were within normal limits. Carbon dioxide combining power was 65.5 volume per cent. Blood pH was not done. For eight consecutive days, the patient was given 80 units of corticotropin intramuscularly with no effect on her total eosinophilic count. Two days later, 25 units of corticotropin produced a drop from 88 to 22 per cu. mm. after four hours. The initial and subsequent administration of corticotropin produced no clinical response.

The patient's further course was uneventful and essentially stable until December 23, 1954, when sudden cyanosis and rapidly increasing tachypnea were noted. Despite intensive supportive measures, the patient died three hours after the onset of these changes.

The autopsy was performed fifteen hours after death. Only the pertinent findings will be described.

Gross Description

There was marked cyanosis of the lips, oral mucosa, fingernails and toenails.

Lungs: All lobes were heavier than normal and subcrepitant to noncrepitant and had a meaty, rubbery consistency. Their cut surface was rusty to purple brown. The right and left upper lobes showed considerable edema. The trachea and major bronchial branches contained tenacious, hemorrhagic mucoid material. The bronchi leading to both lower

and right middle lobes and their secondary and tertiary branches were cylindrically dilated and could be dissected almost to the pleural surfaces. Both main branches of the right and left pulmonary arteries were occluded by an ante-mortem clot. The intima of the main arterial branches was smooth; however, the smaller branches showed numerous atheromatous plaques of the intima.

Heart: Both right atrium and ventricle were markedly dilated. The average thickness of the right ventricle measured 0.8 cm. The right auricular appendage contained a thrombus which was adherent to the wall. Thickness of the left ventricular wall averaged 1.2 cm.

Adrenals: The cortex measured less than 0.1 cm. and appeared light yellow, mottled with darker brown areas.

Microscopic Description

Lungs: Multiple sections taken from all lobes of both lungs showed marked pathologic changes, varying from very slight to complete obliteration of the lung architecture. The minor changes consisted of moderate accentuation of the interlobular and interalveolar septa, showing fibroblastic proliferation, mild round cell infiltration, marked capillary proliferation, and congestion (figure 4). In the areas of more advanced changes, the lobular architecture could not be made out, and there was marked thickening of the interalveolar tissue with formation of dense fibrous tissue, showing mild to moderate round cell infiltration (figure 5). The bronchioles were dilated and in places the mucosa was ulcerated. Marked capillary proliferation and congestion were noted. The arterioles and medium-sized arteries showed hypertrophy of the entire wall, with marked intimal proliferation and hyalinization in the medium-sized and larger vessels, with subsequent narrowing of the lumen. Several medium-sized veins and arteries were occluded by organized thrombi.

A Masson stain revealed marked proliferation of smooth muscle fibers in dilated bronchioles throughout the fibrotic areas (figure 6). The collagen was markedly increased. A reticulum stain (figure 7) revealed marked vascular proliferation in the thickened septa and throughout the increased fibrous tissue. The elastic fibers were increased and frayed in many areas. The pleura was likewise thickened and showed round cell infiltration. Comparison of the lung sections from the biopsy and the autopsy material showed essentially the same picture, although no special stains had been done on the biopsy tissue.

Adrenals (figure 8)*:* Several sections showed marked atrophy of the outer glomerular layer of the adrenals with complete liquid depletion. The outermost portions of the fascicular layer, likewise, showed lipoid depletion, while the inner fascicular layer and the reticular layer showed adequate lipoid content. There were focal areas of lipoid depletion. The width of the cortex was decreased.

Hypophysis showed focal areas where the number of basophils appeared to be increased.

Pathologic Considerations

This case represents a form of diffuse interstitial pulmonary fibrosis and belongs in the group of cases first described by Hamman and Rich (10) as "acute diffuse interstitial fibrosis." Subsequent experience and reports have shown that this is not necessarily a homogeneous group. If the eponym is to be used, it is better therefore to designate these lesions as Hamman-Rich syndrome rather than Hamman-Rich disease. Recently, Golden and Bronk (2) described a progressive diffuse reticuloangiotic type of interstitial pulmonary fibrosis which is characterized by marked capillary proliferation and reticulum hyperplasia and an increase of collagen of varying degree.

The present case presents the markedly increased vascularity and collagenization with little or no interstitial inflammatory reaction, but it is also remarkable for the proliferation of smooth muscle fibers. This muscular hyperplasia in chronic pulmonary lesions has been emphasized by Rubinstein and associates (11) and Liebow and co-workers (12) and seems to be a secondary phenomenon in fibrosing lesions of different etiology.

The bronchiectasis and bronchiolectasis in the right lower and middle lobes observed

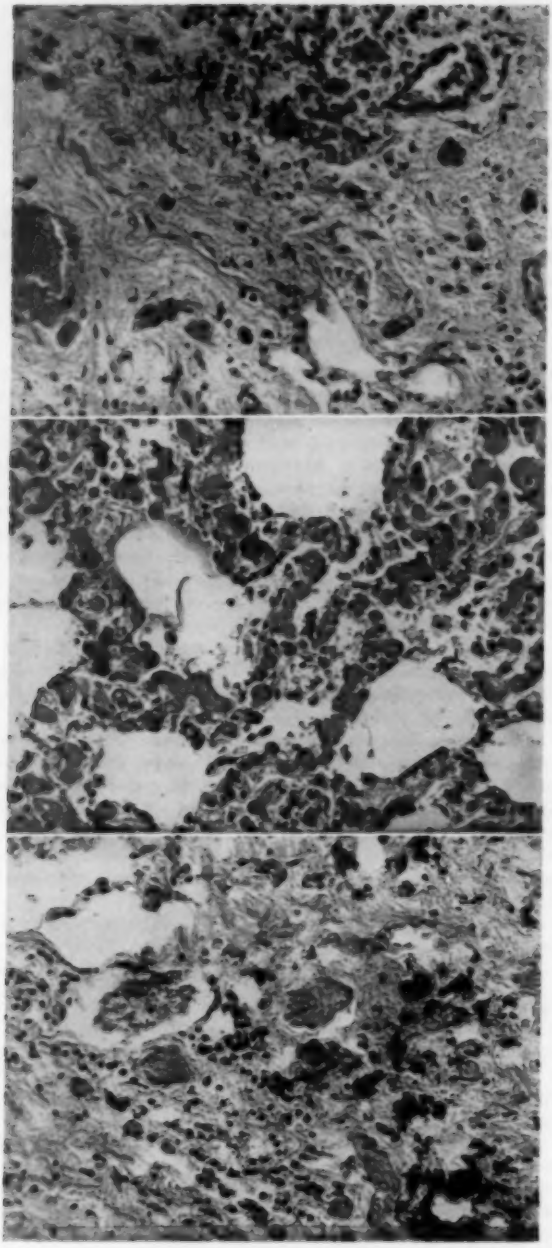

FIG. 4*a* (Top). Specimen obtained at autopsy showing advanced fibrosis with residual alveolar pattern.

FIG. 4*b* (Center). Specimen obtained at autopsy showing early fibrosis with thickening of septa and marked capillary proliferation.

FIG. 5 (Bottom). Specimen obtained at autopsy showing advanced fibrosis.

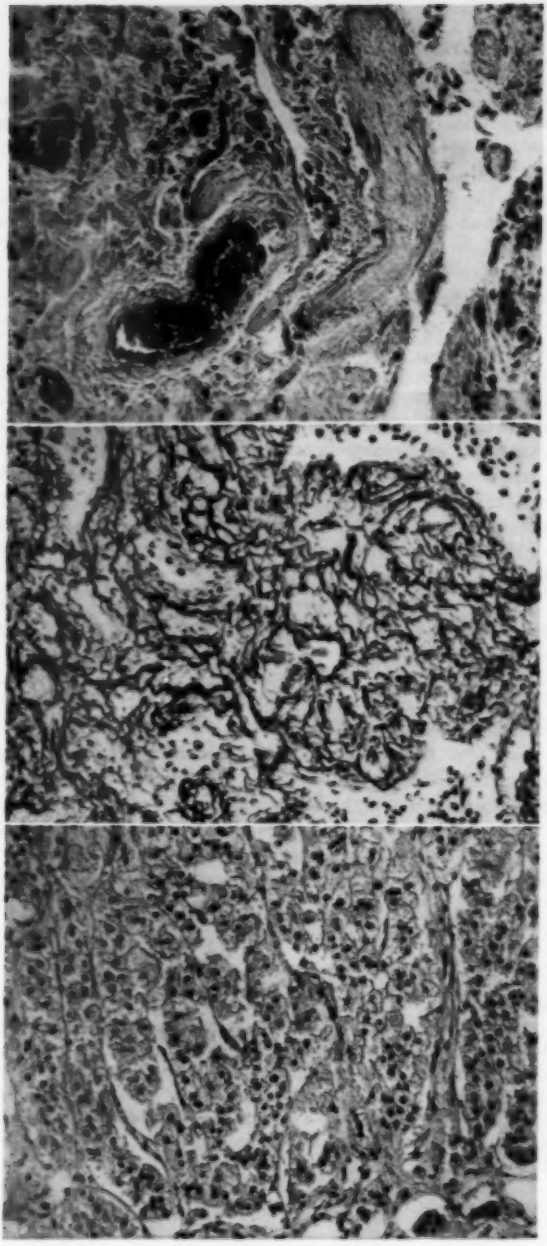

FIG. 6 (Top). Specimen obtained at autopsy. Masson stain showing proliferation of muscle fibers.

FIG. 7 (Center). Specimen obtained at autopsy. Reticulum stain showing marked reticulum and vascular proliferation.

FIG. 8 (Bottom). Specimen obtained at autopsy, showing moderate atrophy of adrenals. This section represents the entire width of the cortex.

in this case seem to be of a local distribution compared with the diffuse fibrosing process involving all lobes, and they are not considered to be of pathogenetic significance.

Discussion

The experience in the present case provides partial answers to certain inevitable questions. As to the arrest of the fibrosing element, assuming that the biopsy specimen represents fairly the condition of the remainder of the lungs three and a half years before death, it seems that the fibrotic changes were neither reversed nor arrested. Parenthetically, it may also be concluded that, if empirical therapy is started with cortisone, such therapy would not impair the diagnostic features of the histologic picture if a lung biopsy were to be done at a later date.

Unfortunately, pulmonary function studies were not available, but several determinations of the vital capacity were in the neighborhood of 1,100 to 1,200 cc. It is a fairly safe assumption that the marked improvement originally noted was not due to any change in total pulmonary capacity or its components, but was more likely due to an improvement of the alveolar-capillary block (9) or the induction of a euphoric state. That the latter is not the sole explanation is suggested by the observation that the clubbing of the fingers became more pronounced and painful when the dose of cortisone was reduced below the level required for symptomatic relief of the cough and dyspnea and was relieved when the dose was increased.

The danger of sudden cessation of cortisone therapy in the Hamman-Rich syndrome has been emphasized. In this instance, the patient's voluntary discontinuance of treatment demonstrated that this danger is not inevitable.

Summary

A case of diffuse interstitial pulmonary fibrosis is presented in which the patient survived for four years after the symptomatic onset, and for three years and five months after the establishment of the diagnosis by lung biopsy. Cortisone therapy of more than two years' duration produced symptomatic and subjective functional improvement but no reversal of the roentgenographic image of the disease. The gross and microscopic features of the lung and pathology are described. Comparison of the surgical biopsy and bronchoscopic specimens shows no appreciable effect of the prolonged steroid therapy on the pulmonary process.

Sumario

Cortisonoterapia in Sindrome de Hamman-Rich

Preséntase un case de fibrosis pulmonar intersticial difusa, en que el enfermo sobrevivió cuatro años la iniciación sintomática y tres años y cinco meses el establecimiento del diagnóstico por la biopsia pulmonar. La cortisonoterapia de más de dos años de duración produjo mejoría funcional, sintomática y subjectiva, pero no viraje de la imagen radiográfica de la enfermedad. Se describen las características macro- y microscópicas del pulmón y de la histopatología. Una comparación de los ejemplares obtenidos con la biopsia quirúrgica y la broncoscopia no revela ningún efecto apreciable de la prolongada esteroidoterapia sobre el proceso pulmonar.

Resume

Cortisonothérapie dans le Syndrome de Hamman-Rich

Présentation d'un cas de sclérose interstitielle pulmonaire diffuse où la survie fut de quatre ans après l'apparition des symptômes et de trois ans et cinq mois après le diagnostic établi par biopsie pulmonaire. Le traitement par la cortisone pendant plus de deux ans

a procuré une amélioration des symptômes et de la fonction, sans modification des aspects radiologiques de la maladie. Les caractères macroscopiques et microscopiques du poumon ainsi que les manifestations pathologiques sont décrites. La comparaison des fragments prélevés par biopsie chirurgicale ou bronchocopique ne révèle pas d'effets notables de la stéroïdothérapie sur le processus pulmonaire.

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HYPERSENSITIVITY REACTIONS DUE TO PARA-AMINOSALICYLIC ACID[1]

Review of the Literature and Report of a Case Showing Cholangiolitic Hepatitis

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INTRODUCTION

With the increase in the number of drugs in the physician's armamentarium in almost geometric proportions in recent years, it is not surprising that ever increasing numbers of hypersensitivity reactions to these drugs are appearing. The reticuloendothelial system is commonly involved in such reactions, and in some cases hepatic manifestations have dominated the clinical picture. In these instances, the differential diagnosis of other forms of hepatitis has been necessary.

As early as 1940, such hepatic manifestations of hypersensitivity were appreciated. Hanger and Gutman (1) reported six cases of post-asphenamine jaundice, unlike the usual toxic hepatitis from arsenicals. They considered the jaundice to be due to hypersensitivity on the basis of a sensitizing dose, rash, arthralgia, edema, eosinophilia, pruritis, icterus, and liver biopsy findings in all cases of pericholangitis and bile thrombi in the finer biliary radicals. Peters (2) later discussed what he considered to be an allergic reaction to arsenical therapy of syphilis which he called "ninth day erythema." In addition to the dermatologic reaction, 13 per cent manifested "hepatitis." If therapy was continued, 70 per cent of the patients had further reactions, 53 per cent of a serious delayed type. This fact, plus the presence of eosinophilia and a definite incubation period, led the author to postulate an antigen-antibody reaction producing the allergic manifestations. Other drugs known to cause cholangiolitic hepatitis have been reported. Buck and Kyle (3) reported three patients who developed jaundice during the course of sublingual methyltestosterone. Werner and associates (4) in 1950 cited seven cases of methyltestosterone icterus. In a recent report by Nelson (5) of two cases of hepatitis from carbarsone, the findings in one suggest cholangiolitic hepatitis on a hypersensitivity basis. Gargill and Lesser (6) cite two cases of jaundice during thiouracil therapy. Both were explored and liver biopsy specimens obtained at operation showed bile plugs and pericholangitis.

Numerous reports have appeared on chlorpromazine jaundice (7–12). The clinical picture suggests hypersensitivity: malaise and fever of abrupt onset with eosinophilia and jaundice characterized by normal flocculation tests and serum proteins, hypercholesterolemia, and elevated alkaline phosphatase. Histopathologic examination of liver sections in this condition have usually shown dark brown areas of canaliculi plugged with thrombi, especially numerous around the central veins, with no cellular reaction around these areas.

Para-aminosalicylic acid (PAS), an antituberculous drug introduced in Sweden by Lehman in 1946 and widely used since 1948, has been responsible for hypersensitivity reactions of significant proportions in approximately 0.3 per cent of those taking the drug (13). Jaundice, as a manifestation of PAS hypersensitivity, is infrequently reported and the pathology has been ill defined in previous reports.

The following case report illustrates a nonfatal hypersensitivity reaction to PAS, liver biopsy showing a cholangiolitic type of hepatitis. When biopsy repeated one month later

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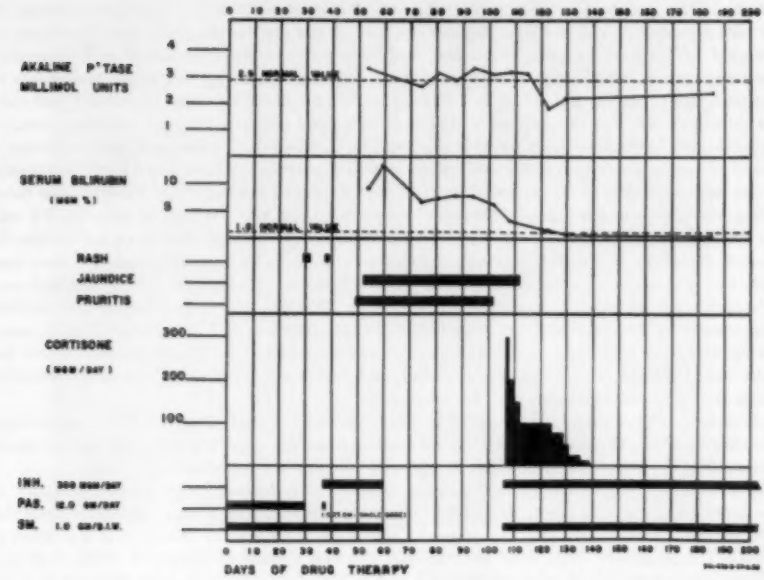

FIG. 1. Clinical course of PAS hepatitis. Liver biopsies were done on the sixty-second and one hundred and sixth days after onset of drug therapy (the thirty-second and seventy-sixth days after onset of rash).

INH—isoniazid
PAS—para-aminosalicylic acid
SM—streptomycin

showed a progression of the lesion, cortisone was used with rapid defervescence of the clinical and laboratory evidence of hepatotoxicity and, incidentally, continued clearing of the pulmonary tuberculosis.

CASE REPORT (Figure 1)

A 26-year-old, white male was hospitalized on September 24, 1955, with moderately advanced pulmonary tuberculosis of the right upper lobe. Sputum smears were positive for acid-fast bacilli and subsequent cultures were also positive. On September 30, 1955, the patient was started on therapy with streptomycin, 1.0 gm. twice weekly, and PAS, 12 gm. daily. An initial hemogram, including leukocyte and differential counts, was within normal limits. The sedimentation rate was 11 mm. per hour. Other laboratory work was noncontributory. The patient was afebrile. His weight increased from 132 pounds on admission to 141 pounds on October 30. On October 30, the patient developed a diffuse, dry, pruritic, erythematous macular rash over the entire trunk and extremities approximately four hours after he received a streptomycin injection. PAS was discontinued at that time and therapy with pyribenzamine and calamine lotion was instituted. Streptomycin was not discontinued inasmuch as it would be four days until the next injection. Subsequent streptomycin injections caused no reaction. The rash subsided over a period of two days, but slight itching persisted. On November 2, the leukocyte count was 6,050 with 43 per cent neutrophils, 41 per cent lymphocytes, 8 per cent monocytes, 7 per cent eosinophils, and 1 per cent basophils. Three of the lymphocytes were noted to be atypical. On November 6, a single dose of 0.25 gm. of PAS was administered orally and within twenty-four hours the rash had

returned. The leukocyte count on November 7 was 5,850 with 48 per cent neutrophils, 39 per cent lymphocytes, 3 per cent monocytes, and 10 per cent eosinophils. On November 7, isoniazid, 300 mg. daily, was instituted and streptomycin was continued as previously. The temperature remained normal except for a single elevation to 99.2°F. on November 9. The rash again rapidly subsided, but itching persisted. On November 22, scleral and skin icterus was noted. The patient continued to have a good appetite without nausea or vomiting, although inquiry at this time revealed that he had had dark urine and light stools for a period of four or five days. Liver and spleen were not palpable. There was a slight erythema of the neck and skin of the extremities, but no significant adenopathy. At this time total serum bilirubin was 9.9 mg. per 100 ml. (1 minute–4.8 mg. per 100 ml.; 30 minutes–7.1 mg. per 100 ml.). Alkaline phosphatase was 3.4 millimol units (normal—less than 2.9 millimol); thymol turbidity, 1.9 units; cephalin flocculation, 1-plus. Heterophil antibody titer was 1:28, but was negative after both guinea pig and beef cell absorption. Urine bile and urobilinogen were recorded as negative on November 25, 1955, but on November 28 bilirubin was present in the urine and urine urobilinogen was positive at 1:10 dilution. On November 23, the liver edge was palpable, smooth, and nontender one fingerbreadth below the right costal margin. The spleen was definitely palpable by November 25, two fingerbreadths below the left costal margin, and was nontender.

Jaundice persisted and, although it was believed by the staff that this was a sensitivity reaction to PAS, it was advised that all antituberculous chemotherapy be discontinued temporarily to avoid confusion. All drugs were stopped on November 29.

A liver biopsy on November 30 yielded tissue which demonstrated no abnormality of the liver parenchymal cells. There was considerable staining of the reticuloendothelial cells within the sinusoids with bile pigment, and a small amount of fibrosis and a number of chronic inflammatory cells were seen around the bile canaliculi (figure 2). This was considered characteristic of cholangiolitis.

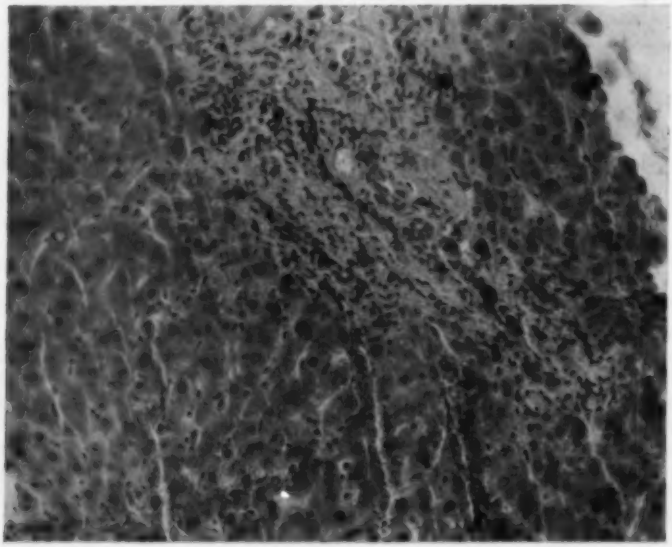

FIG. 2. Liver biopsy on November 30, 1955. There is bile staining of reticuloendothelial cells, slight fibrosis, and chronic inflammatory reaction around bile canaliculi. Liver parenchymal cells are intact.

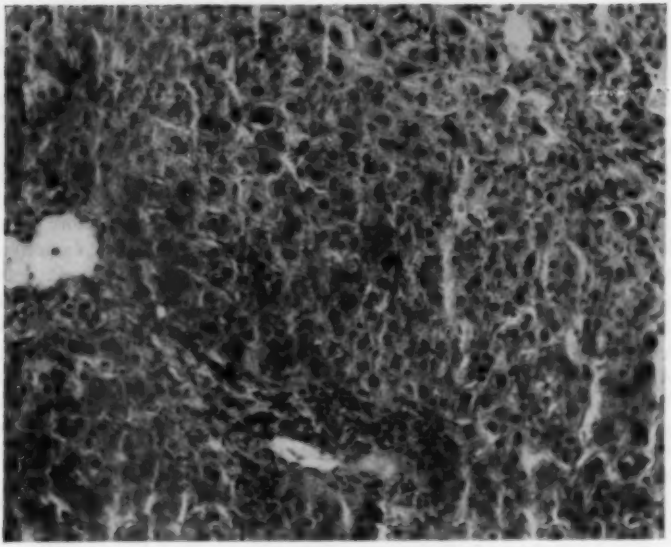

FIG. 3. Liver biopsy on January 13, 1956. There is less marked inflammatory reaction in the portal zones, and increased bile staining in the central lobular areas, compared with the previous biopsy. No evidence of hepatic cell necrosis is seen.

Serial laboratory studies revealed persistent bilirubinuria, but urine urobilinogen was never positive above a dilution of 1:10. Serum bilirubin and alkaline phosphatase remained elevated, but all other tests of liver function, including serum cholesterol and esters, thymol turbidity, cephalin flocculation, and serum proteins and albumin-globulin ratio, remained within normal limits throughout the month of December. The liver remained palpable, although the spleen was no longer palpable after December 19. The patient lost twelve pounds of weight between November 13, 1955, and January 8, 1956.

On January 13, a second liver biopsy was done. The most striking pathologic change noted was marked bile stasis in the canaliculi. This was most noted in the central lobular areas. There were no bile thrombi in the larger bile ducts of the portal zones. Some bile pigment was seen in the Kupffer cells. The general architecture of the liver was preserved and the hepatic cells showed no evidence of necrosis. However, in some areas the nuclei were variable in size, many of them being unusually large, and some cells were binucleate. The portal zones showed only mild infiltration with lymphocytes. There was no notable degree of bile duct proliferation (figure 3). These changes were interpreted as suggesting an intralobular biliary obstruction (bile stasis and chronic periportal hepatitis). On January 16, 1956, the patient was re-started on antituberculous chemotherapy with streptomycin, 1.0 gm. twice weekly, and isoniazid, 300 mg. daily in divided doses. He was simultaneously started on oral cortisone, beginning with 300 mg. per day, and thereafter gradually decreasing doses over a thirty-one day period. Serum bilirubin subsided to 1.3 mg. per 100 ml. by January 31, by which time the alkaline phosphatase was 1.8 millimol units. Thereafter, all liver function studies were within normal limits. Bromsulphalein retention, following subsidence of jaundice, was normal. There was a steady gain in weight and since the middle of April, 1956, weight has been stable at 145 pounds. Chest roentgenograms continued to show improvement, beginning in November, 1955, and continuing throughout January, 1956. Thereafter, chest roentgenograms were stable with only a minimal residual of disease. Cortisone was discontinued on February 16, 1956.

Review of the Literature

True hypersensitivity to PAS has been of two types. Mild reactions, such as coryza, headaches, lacrimation, conjunctivitis, spasmodic coughing, wheezing and fever, are often observed but seldom reported. The more serious types are manifested by hemorrhagic disease (14), including purpura and menorrhagia; eosinophilic pneumonitis (15, 16); skin eruptions (usually maculopapular or scarletiniform), with eosinophilia (17, 18); central nervous system changes, sometimes mimicking tuberculous meningitis (19); and hepatitis.

Sporadic reports of hepatotoxicity first appeared in the British literature (20–22). In the first comprehensive review of the subject in 1954, Dixon (23) collected from the American and British literature a total of twenty-six cases of jaundice due to PAS. Most of these patients received between 15 and 20 gm. of PAS daily, a dose customarily used by the British. A typical clinical syndrome was apparent in these cases. Manifestation of hypersensitivity was heralded by sudden onset of pyrexia and rash, jaundice appearing two to sixteen days later. In most cases, jaundice appeared from twenty-two to forty-six days after therapy was started, and the total administration of the drug ranged from 163 to 820 gm. Eosinophilia, often transient, was apparent in eighteen of twenty-one patients in which differential counts were performed, and other manifestations of hypersensitivity were variously present: lymphadenopathy, angioedema, splenomegaly, arthralgias, glossitis, and stomatitis. Liver function studies were not recorded in most cases. In those cases in which they were recorded, 3-plus cephalin flocculation in two; thymol turbidity, 6 units, in one; and elevated alkaline phosphatase in one (28.6 King-Armstrong units), were found. Three of the twenty-six cases were fatal, although one patient was severely dehydrated, one had concomitant agranulocytosis, and one had intractable hypopotassemia. Two patients at autopsy showed extensive severe parenchymal hepatic damage.

Later, as more of the drug was used in the United States, well-documented cases of hepatotoxicity appeared. Friedman (15) cited a case of 20 per cent eosinophilia, 11 per cent bromsulphalein retention, and total serum bilirubin, 1.7 mg. per 100 ml. Hayes and Weiss (24) cited ten reactions in 255 patients, two of whom presented the syndrome described by Dixon in England of fever, rash, and eosinophilia, followed by icterus. Liver function tests recorded in one of these patients showed an icteric index of 100, thymol turbidity of 57 units, and a reversed albumin-globulin ratio, all of which reverted to normal in one month. Paine (25) reported six cases, one with rash, fever, monocytosis but negative heterophil, eosinophilia, and evidence of liver involvement: abnormal cephalin flocculation, slightly elevated alkaline phosphatase, and elevated direct bilirubin.

In a series of two articles, Cannemeyer and associates (13, 26) analyzed fourteen hypersensitivity reactions in approximately 5,000 patients taking PAS. All of the fourteen patients developed a reaction fifteen to forty-three days after the start of therapy, characterized by fever, rash, and pruritis, with cervical lymphadenopathy in eleven patients, enlarged tender liver in five patients, splenomegaly in three, and clinical icterus in two. In a comprehensive laboratory analysis, the following characteristics of the reaction were noted: relative or absolute lymphocytosis with atypical forms and an eosinophilia, with uniformly negative cold agglutination and Paul-Bunnell reactions, and frequently positive patch tests to PAS. In those patients with hepatomegaly, splenomegaly, and jaundice, alkaline phosphatase values of 13.7, 21.4, 28.0, and 52.6 units were found. Other abnormal liver profile studies included thymol turbidity, 8–24 units in six patients; serum bilirubin, 1.9 to 6.4 mg. per 100 ml. in four patients; cephalin flocculation, 3–4-plus in eight patients; bromsulphalein retention, 10, 15, 20, and 30 per 100 ml. in four cases. All segments of the liver profile and plasma proteins tended to approach normal within one to two months of onset.

The seriousness of such reactions is attested to by the fact that five and possibly six fatal cases of hepatitis from PAS have been reported. Tyrell's case is cited by Cuthbert (22), but no details are given. Landsborough (21) describes a case of a Chinese female with active tuberculosis treated with PAS alone, 15 gm. every day, who, after seventeen days of therapy, developed anorexia, fever, nontender hepatomegaly, a morbilliform rash, and petechiae within thirteen days. At this time the drug was stopped. However, deep jaundice, subconjunctival hemorrhages, coma, and death occurred twenty-seven days after the reaction began. No liver studies were reported; however, bilirubinuria was noted. No autopsy was performed. Muri (27) reported the case of a 41-year-old male with cavitary tuberculosis treated with 17 gm. of PAS daily, who manifested a rash on the eighth day of therapy, then icterus and a liver palpable 10 cm. below the right costal margin two days later. The leukocyte count was 5,300 with 67 per cent lymphocytes, all atypical. Despite supportive measures, the patient died twenty-one days after PAS was begun, the drug having been stopped seven days after manifestations of toxicity appeared. This patient received barbiturates and acetylsalicylic acid (allypropynal, 200 mg. on the second, fourth, ninth, and tenth days, and barbacetin on the seventh hospital day). Whether these contributed to the patient's demise is only a matter of conjecture.

Hansen and Cleve (28) reported a fatal case of hypersensitivity in a patient with miliary tuberculosis who received streptomycin and PAS. The patient manifested a temperature rise following each injection of streptomycin every third day. Even after discontinuing this, severe conjunctivitis, lymphadenopathy, anorexia, and cough persisted and, two days later, PAS was discontinued. One week later the patient had improved and PAS was reinstituted, with rapid onset of fever to 104°F., stupor, rash, and 23 per cent eosinophilia. Despite discontinuing all medication four days later, the patient died after another four-day interval. Icterus was apparent only at death. At autopsy, massive hepatic necrosis was found. While in all probability PAS was the offending drug, streptomycin may have played some part in this case.

Another fatal case of PAS hepatitis showing widespread subacute hepatic cell necrosis was reported by Allen and associates (29). However, tubercles were also found in the liver, spleen, and brain. Thus, miliary tuberculosis with hepatic involvement was present, although the clinical picture suggested PAS hypersensitivity. Another case of miliary tuberculosis with histologic evidence of tuberculous involvement of the lungs, myocardium, kidneys, pancreas, spleen, diaphragm, brain, uterus, and liver was reported by Bellamy and co-workers (30). The patient had fever, rash, and eosinophilia three weeks after beginning PAS, which was then discontinued. However, two weeks later, despite persistent rash and fever, PAS was re-started and the patient had a rapidly fulminating course with jaundice and death in five days. At autopsy, the liver showed hard and soft tubercles with widespread necrosis.

Discussion

The case reported in the present paper demonstrates cholangiolitic hepatitis as part of a generalized hypersensitivity reaction to PAS, based on the clinical findings, liver function tests, serial liver biopsies, course, and response to cortisone. To the knowledge of the present writers, this is the first report of histologic examination of liver tissue in a nonfatal case of PAS jaundice.[2] While patch tests to PAS have been positive in about 30 per cent

[2] Since preparation of this report, a case of exfoliative dermatitis and cholangiolitic hepatitis, proved by liver biopsy, has been reported. The patient showed skin sensitivity to both streptomycin and PAS. Convalescence was slow, with prolonged evidence of bromsulphalein retention (Quart. Progress Reports of VA-AF Study on the Chemotherapy of Tuberculosis, 1956, *11*, 27).

of reported cases in one series (23), the test in the present case was negative. An oral test dose of 0.25 gm. of PAS, however, resulted in reappearance of the patient's rash, thus implicating this drug. The fact that the patient had not received any intravenous infusions in the preceding six months, had been under isolation, and had not come in contact with anyone with jaundice for the previous two months, the absence of constitutional symptoms, the negative flocculation tests and, above all, the histopathologic findings rule out viral hepatitis in this case.

The fact that fatalities can occur with cholangiolitic hepatitis should serve as a warning to discontinue PAS at the first major sign of toxicity: fever, rash, pulmonary infiltrate, arthralgias, or signs of liver dysfunction. It is suggested that all patients on PAS have a periodic estimation of the icteric index or serum bilirubin. Any elevation of the icteric index or serum bilirubin should be confirmed by a complete liver battery, with special attention to the alkaline phosphatase.

Densitization to PAS, starting one to two months after the hypersensitivity reaction has completely subsided, has been successful (12). However, with isoniazid now available, and with other drugs such as viomycin and cycloserine showing promise, desensitization to PAS should rarely be necessary. It would not seem wise to substitute pyrazinamide, since this drug has been reported to be hepatotoxic. Substitution of isoniazid in the present case was successful. On the basis of its use in other hypersensitivity reactions and in other forms of cholangiolitic hepatitis, cortisone and/or corticotropin would seem to be the treatment of choice, provided adequate chemotherapy is used concurrently.

Summary

A hypersensitivity reaction to PAS characterized by cholangiolitic hepatitis, confirmed by serial liver biopsies and treated by cortisone, is presented. The available literature on PAS hepatitis is reviewed, and other drugs which may cause similar reactions are cited. A rationale of treatment is outlined.

Sumario

Reacciones Hipersensibles Debidas al Acido Para-Aminosalicílico: Repaso de la Literatura y Presentación de un Caso con Hepatitis Colangiolítica

Descríbese una reacción de hipersensibilidad al PAS, caracterizada por hepatitis colangiolítica, confirmada por biopsias hepáticas en serie, y tratada con cortisona. Repásase la literatura accesible y se citan otras drogas que pueden ocasionar reacciones semejantes.

Resume

Réactions d'hypersensibilité dues à l'acide para-aminosalicylique: Compte-rendu de la littérature et observation d'un cas présentant une hépatite cholangiolytique

Rapport sur un cas de réaction d'hypersensibilité au PAS caractérisée par une hépatite avec angiocholite confirmée par des biopsies hépatiques en série et traitée par la cortisone. La littérature sur l'hépatite due au PAS est passée en revue et les auteurs citent d'autres drogues pouvant causer des réactions semblables. Les raisons justifiant le traitement sont exposées.

Acknowledgments

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ACTIVATION OF TUBERCULOSIS DURING PREDNISONE THERAPY[1]

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INTRODUCTION

There have been reports of either the appearance of miliary tuberculosis (1–3) or the reactivation with lymphatic spread of an old, healed tuberculous lesion (4) following corticotropin or cortisone therapy in patients with disseminated lupus erythematosus. The following case is the first such occurrence during prednisone therapy known to the present writers.

CASE REPORT

D. M., a 75-year-old white female, was hospitalized at the University of Chicago Clinics on February 13, 1955, complaining of pain in the wrists, shoulders, and knees of twelve months' duration. During most of this time she felt feverish and the wrists had been swollen for a brief period. For six months her appetite had been poor and she had lost 25 pounds in weight. She had felt nauseated but had never vomited, and the bowel function was relatively normal. Two months previous to admission the patient had had an episode of pain, redness, and swelling in the eyes. For one month she had noted dyspnea on exertion and a sharp pain in the left side of her chest that was aggravated by respiration. She denied any cough, sputum, hemoptysis, night sweats, orthopnea, ankle swelling, or urinary or neurologic symptoms. Her past health had been good except for episodes of mild polyarthritis in 1939 and 1948, accompanied by episcleritis in 1939. A diagnosis of nontoxic nodular goiter had been made in 1952. There was no known tuberculous infection or contact, and no family history of diabetes mellitus was disclosed.

On examination the patient appeared both acutely and chronically ill with a temperature of 38.6°C.; pulse, 120 per minute; blood pressure of 120 systolic, 60 diastolic in mm. of mercury; respiration, 26 per minute. There was a leukoplakic lesion in the mouth, but there was no evidence of inflammation in the eyes, ears, nose, or throat. A multinodular thyroid gland was enlarged to twice the normal size. No skin rash or lymphadenopathy was noted. The left diaphragm moved poorly and coarse rales were audible at both lung bases. The heart was normal in size with a regular rhythm, sounds of good quality, no murmur, rub, or gallop. No organs, masses, or areas of tenderness were palpable in the abdomen. Pelvic and rectal examinations were negative except for evidence of atrophic vaginitis. There was no swelling, warmth, tenderness, or deformity of any joint. Muscle strength was good except for some weakness of the fingers. There was no meningismus; cranial nerves were intact; and deep tendon reflexes were equal and active.

The blood hemoglobin was 12.0 gm. per 100 ml., and the erythrocyte count was 3,950,000 per cu. mm. The leukocyte count was 6,700 per cu. mm. with 86 per cent neutrophils and 14 per cent lymphocytes. The erythrocyte sedimentation rate was 44 mm. per hour by the Wintrobe method. A urine specimen was found to have a specific gravity of 1.022 and to be free of albumin or reducing substance. Microscopic examination of the urinary sediment after centrifugation showed 0 to 2 erythrocytes and 4 to 6 leukocytes per high power field. A serologic test for syphilis (Kahn) was negative. Three stool specimens failed to give a benzedine reaction for occult blood. Total plasma protein was 6.9 gm. per 100 ml. with albumin 2.8 gm. per 100 ml. and globulin 4.1 gm. per 100 ml. A serum specimen showed a 2-plus test for C-reactive protein. Total serum bilirubin was 0.4 mg. per 100 ml.; alkaline phosphatase, 2.2; Bodansky units and cephalin flocculation, 3-plus. The blood urea nitrogen

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[1] From the University of Chicago Clinics, Chicago 37, Illinois.

was 8.0 mg. per 100 ml. An intradermal test with Old Tuberculin (OT), diluted 1:10,000, was negative, as had been the case in 1939.

Three blood cultures were sterile and a catheterized urine culture showed *E. coli*. A chest roentgenogram showed slight obliteration of both costophrenic angles, slight scarring in the right apex, and calcified nodules in both hilar regions and in the right lower lobe. There was no significant change on comparison with a chest film taken in 1939. Roentgenographic examination of the esophagus, stomach, and duodenum was normal. Five lupus-erythematosus preparations on peripheral blood were strongly positive.

During the first week in the hospital, the patient's condition worsened, with increasing fever, weakness, anorexia, dyspnea, tachycardia, and cardiac enlargement. An electrocardiogram showed sinus tachycardia, S-T segment depression in leads I, II, and V_L, low T waves in I, II, and V_5, and inverted T in III and V_4.

A diagnosis of acute disseminated lupus erythematosus was made on the basis of the gravity of the patient's general condition, multiple system involvement (joints, pleura, heart, and kidneys), reversal of the plasma albumin-globulin ratio, and positive lupus-erythematosus preparations. She was given Digilanid®, 5.33 mg. in five days, and was started on hydrocortisone, 100 mg. daily. Fever subsided within twenty-four hours but the dosage was increased to 160 mg. because of an otherwise unsatisfactory response. Marked hyperglycemia and glucosuria appeared. Prednisone, 50 mg. daily, was substituted for the hydrocortisone. Neutral Protamine Hagedorn (NPH) insulin, 40 units daily, was given. Thereafter the condition gradually improved and the patient was discharged on the seventy-seventh day on prednisone, 20 mg.; Digilanid, 0.33 mg.; and NPH insulin, 30 units daily.

The patient did poorly during the next twelve months despite continuous prednisone therapy in doses of 17.5 mg. to 25 mg. daily. Leukocyte counts ranged from 3,800 to 8,700 cells per cu. mm. The lupus-erythematosus preparations became negative. Follow-up chest roentgenograms obtained in April and December, 1955, showed no evidence of active pulmonary disease. The patient was again admitted to the hospital on May 1, 1956, after three months of fever and weakness that would not respond to prednisone, 40 mg. daily. On examination, temperature was 39.2°C., pulse, 120 per minute; blood pressure, 110 systolic and 50 diastolic in mm. of mercury; and respirations, 24 per minute. There was no skin rash or lymphadenopathy. The findings in the head, eyes, ears, nose, and throat were not abnormal. Scattered rales and bronchial breath sounds were heard over both lungs. The heart was markedly enlarged and a systolic murmur was audible in the pulmonic area. Examination of the abdomen and the extremities was negative.

Examination of the blood showed hemoglobin, 11.8 gm. per 100 ml.; leukocytes, 5,200 per cu. mm., with 86 per cent neutrophils, 12 per cent lymphocytes, and 2 per cent monocytes. Urinalysis revealed a specific gravity of 1.014; albumin, 1-plus; sugar, negative; 10 erythrocytes and 15 leukocytes per high power field in a centrifuged specimen. The serum sodium was 122 mE per liter; chloride, 86 mE per liter; and carbon dioxide, 30.6 millimols per liter. The plasma albumin was 2.5 gm. per 100 ml.; and globulin, 3.8 gm. per 100 ml. The blood urea nitrogen was 19.2 mg. per 100 ml. A blood culture was sterile; sputum culture showed *Staph. aureus*; and urine culture, *E. coli*. A chest roentgenogram showed patchy areas of diffuse infiltrate throughout both lung fields and a nodular density in the right base.

A diagnosis of pulmonary disease due to disseminated lupus erythematosus was made, but the patient failed to respond to intravenous hydrocortisone, 100 mg. daily. The question of superimposed staphylococcal pneumonia was raised, and tetracycline, 1.0 gm., was added to the daily regimen. This was followed by a subsidence of fever but increasing physical and roentgenographic signs of consolidation of both lower lobes. A skin test (OT, 1:10,000) was again negative, but on the eleventh hospital day large numbers of acid-fast bacilli were found in the sputum. The patient was desperately ill at this time. Prednisone was continued in a dose of 60 mg. daily, and on the fifteenth hospital day she was started on isoniazid, 300 mg.; streptomycin, 1.0 gm.; and para-aminosalicylic acid (PAS), 10.0 gm. Her temperature rose to 40.6°C.; tube feeding had to be instituted; there was evidence of cerebral disturbance. She died on the twentieth hospital day.

Autopsy report: The pleural and pericardial cavities were obliterated by dense fibrous adhesions. There were large, poorly circumscribed, sometimes confluent, areas of caseous necrosis throughout all lung lobes with collections of large macrophages at the periphery but no epithelioid or giant cells and no fibrous reaction. Acid-fast stains showed numerous acid-fast bacilli. In the peribronchial lymph nodes there were also confluent areas of caseous necrosis with epithelioid cells in some places but no giant cells.

Moderate perivascular fibrosis was observed in the left ventricle of the heart as well as some focal calcification in the aortic valve and atheromatous plaques at the base of the aorta. There were no other valvular or endocardial lesions.

Multiple miliary tubercles were seen in the liver and spleen. Those in the spleen were present chiefly as epithelioid and giant cell reactions. A few miliary tubercles were seen in the kidneys, uterus, ovaries, and bone marrow.

The arterioles and small arteries of the spleen showed a loose fibrous perivascular reaction compatible with old disseminated lupus erythematosus. Old depressed cortical scars were seen in both kidneys with lymphocytic infiltration and hyalinized glomeruli. The renal vessels and glomeruli were otherwise not unusual. There were neither "wire loop" lesions of the basement membrane nor hematoxylin bodies. There was an acute peptic ulcer at the pylorus, and an old thrombophlebitis of the left femoral vein was noted. Examination of the brain revealed only mild to moderate cerebral atrophy.

Discussion

The elderly patient in this case presented with an obscure illness affecting diverse organ systems. The possibility of active tuberculosis at the outset seemed unlikely in view of the unchanged appearance of the chest roentgenogram during the interval from 1939 to 1955. The diagnosis of acute disseminated lupus erythematosus and inactive pulmonary tuberculosis seemed a much more likely, and a completely adequate, explanation for the initial findings. Corticotropin and cortisone have been reported to limit inflammation, granulation, and fibrosis, with complicating progression and dissemination (8). It is of interest that positive lupus-erythematosus preparations have been reported in miliary tuberculosis (5–7).

During the fourteen-month period of adrenal cortical hormone therapy the pulmonary lesions were carefully watched. Their unchanged appearance on chest roentgenograms obtained after two months and nine months, respectively, tended to create a false sense of security. When uncontrollable fever recurred, there was a delay in suspecting active tuberculous infection. Predisposing factors that must be considered, in addition to the prednisone therapy, include the disseminated lupus itself, general debility, malnutrition, and diabetes mellitus.

The autopsy findings showed, in addition to the evidence of disseminated lupus erythematosus, a strong suggestion that one or more of these factors had interfered with the usual bodily reaction to tuberculous infection. It remains to be seen whether this phenomenon will be reported with any frequency in patients taking the delta-steroids (prednisone and prednisolone).

Summary

The patient, with a clinical and autopsy diagnosis of disseminated lupus erythematosus, developed a severe fatal disseminated tuberculosis after fourteen months of adrenocortical steroid therapy. During twelve of these months this therapy was prednisone in doses of 17.5 to 25.0 mg. per day. The effect of predisone per se on the activation of tuberculosis is not very clear in this individual case, but it is hoped that subsequent reporting will help to clarify this problem.

Sumario

Activación de la Tuberculosis Durante la Prednisonoterapia

El enfermo, con un diagnóstico clínico y autópsico de lupus eritematoso diseminado, manifestó una granulia grave letal al cabo de catorce meses de terapéutica con esteroides corticosuprarrenales. Durante doce de esos meses, esa terapéutica consistió en prednisona a dosis de 17.5 a 25.0 mg. al día. En este caso específico, no quedó muy en claro el efecto de la prednisona por sí misma sobre la activación de la tuberculosis, pero se espera que estudios ulteriores ayudarán a esclarecer este problema.

Resume

Activation de la tuberculose durant le traitement par la prednisone

Le malade, dont le diagnostic clinique de lupus érythémateux disséminé a été vérifié à l'autopsie, a présenté une tuberculose généralisée fatale au bout de quatorze mois de traitement par les corticostéroïdes. Durant douze de ces mois, c'est la prednisone qui était administrée à la dose de 17, 5 à 25 mg par jour. L'effet de la prednisone en soi, dans l'activation de la tuberculose, n'est pas très clair dans ce cas particulier, mais les auteurs souhaitent que des rapports ultérieurs éclaircissent ce problème.

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NONREACTIVE TUBERCULOSIS[1]

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Introduction

In 1939, Siegmund (1) introduced the term "generalized nonreactive tuberculosis" to describe a fatal form of tuberculosis in which many organs contain miliary foci of necrosis surrounded by normal parenchymal cells. The typical tuberculous cellular response is either completely absent or extremely poor. The lesions are usually replete with tubercle bacilli. This form of tuberculosis was called by Rich and McCordock (2) "acute caseating miliary tuberculosis," and the characteristic loosely formed necrotic lesion seen on autopsy was described as a "soft" tubercle in contrast to the closely knit, epithelioid cell "hard" tubercle observed in ordinary miliary tuberculosis. The lesions are extremely numerous, and the great majority are almost or completely necrotic. In several cases of nonreactive tuberculosis, in addition to small miliary tubercles, more progressive lesions of larger size are seen, up to 2 cm. (3) with a less even distribution. Straub (4) refers to this condition as hematogenous disseminated tuberculosis with larger nodules. In few cases, the macroscopic findings at autopsy resembled Hodgkin's disease.

This peculiar form of tuberculosis, although fortunately rare, is of interest because of its unusual clinical features. Briefly, the clinical course of this condition is that of an acute, overwhelming, generalized infection; less often the syndrome presents as a chronic fever of unknown origin. Usually there are gross, severe changes in the blood picture which may be anemia, polycythemia, agranulocytosis, or a leukemoid reaction. One-fourth of the cases reported are associated with definite pre-existing hematologic disease such as leukemia, myelosclerosis, or Hodgkin's disease. Only about 10 per cent of the patients have a normal blood picture during the illness. The spleen is almost always enlarged; the liver, often. Thus, the name ot typhobacillosis of Landouzy (1891) was used in the German literature to indicate a syndrome, the cause of which was a "toxemic" form of tuberculosis, with a clinical picture resembling typhoid fever in many respects.

O'Brien (3) reviewed the subject in 1954 and found only sixty-six cases in the literature which fulfill all of the criteria mentioned above. He reported eight additional new cases and suggested the term "nonreactive tuberculosis."

The case presented in this report is of special interest in that it originated in a patient with a treated, apparently healed tuberculous lesion in the lung, in whom this syndrome may have been precipitated by a hemotoxic reaction to chlorpromazine

Case Report

The patient was a 62-year-old white single male, admitted to the hospital in 1949 with a history of wheezing respiration, right chest pain, intermittent cough productive of one ounce of sputum per day, and occasional night sweats of approximately one year's duration.

The past history revealed that the patient had had numerous chest roentgenograms in 1929 because of a pulmonary infiltrate which was interpreted at the time as inactive tuberculosis. In 1944, active endobronchial tuberculosis was found by bronchoscopy, but he did not receive any treatment for it. Between 1944 and 1946, the patient was in a mental institution; the psychiatric diagnosis was not known. Shock therapy was given at that time. One sister had died from tuberculosis, and another sister had recovered from pulmonary tuberculosis.

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Physical examination on admission showed a well-developed, well-nourished middle-aged male, moderately dyspneic, who appeared chronically ill. Numerous rhonchi and wheezes were heard in both lung fields. The skin test, OT, 1:1,000, was positive.

The sputum on admission was positive for tubercle bacilli on smear and culture. The hemogram was: hemoglobin, 14.0 gm. per 100 ml.; erythrocyte count, 4,900,000 per cu.mm.; leukocyte count, 11,800 per cu.mm., with normal differential count. The urine was normal. A chest roentgenogram revealed that the bronchovascular markings were increased bilaterally. A soft nodular infiltrate was seen between the left first and third anterior ribs. The right lung was free of parenchymal disease. A few small calcifications were found in both hila.

Following admission, the patient received several short courses of daily streptomycin-PAS therapy between 1949 and 1952. In June, 1951, he had a spontaneous pneumothorax on the left; in December, 1951, a spread of tuberculosis was noted throughout the left lower lung field. In 1953, prolonged chemotherapy with isoniazid, PAS, and bi-weekly streptomycin was started. The patient showed some clinical improvement, but he became very short of breath, which was thought to be at least partly psychogenic in origin. His sputum finally became noninfectious (culture) after February, 1954, and remained so until his death in January, 1956. Serial chest roentgenograms became stationary in 1954 (figure 1), and in January, 1955, all chemotherapy was discontinued. He was kept in the hospital as a

FIG. 1. A representative of roentgenograms of the chest obtained in 1954 and 1955, showing a stable far advanced fibrocaseous tuberculous lesion on the left, with deviation of the trachea and compensatory distention of the right lung.

TABLE 1

HEMATOLOGIC FINDINGS

Date	October, 1953	August, 1954	August, 1955	December 16, 1955	December 28, 1955
Hemoglobin (gm. per 100 ml.)	14.9	13.7	12.1	6.30	9.0
Erythrocytes (millions)	5.06	4.95	4.10	2.62	3.09
Leukocytes per cu. mm.	5,600	4,350	3,300	1,050	600
Segmented polymorphonuclears per cent.	60	54	42	55	40
Nonsegmented polymorphonuclears per cent	7	8	4	4	12
Lymphocytes per cent	29	37	52	41	48
Monocytes per cent	4	—	—	—	—
Eosinophils per cent	—	1	2	—	—
Basophils per cent	—	—	—	—	—
Hematocrit	30	49	41	28	30
Erythrocyte sedimentation rate (Wintrobe)	16	27	30	16	28
Platelets	—	—		141,000	—
Icterus index	—	—	7.5	5	—

custodial case because no suitable home could be found for him due to his mental condition, which was diagnosed by the psychiatric consultant as involutional psychosis in partial remission.

In July, 1955, the patient was started on chlorpromazine (Thorazine®), 200 mg. daily, in the hope of improving his mental condition.

The patient had a leukocyte count of 5,600 per cu.mm. in 1953; 4,900 per cu.mm. in August, 1954; and 3,300 per cu.mm. in August, 1955 (table 1). He was doing well clinically until December, 1955, at which time he complained of a sudden onset of malaise, and his temperature rose to 101° F. Physical examination revealed an enlarged spleen (figure 2). An emergency hemogram showed a hemoglobin of 6.3 gm. per 100 ml., an erythrocyte count of 2,600,000 per cu.mm., a leukocyte count of 1,050 per cu.mm., with 55 per cent segmented and 4 per cent nonsegmented polymorphonuclears. His sputum did not show acid-fast bacilli on either smear or culture. The urinalysis was within normal limits. A chest roentgenogram showed the same stable lesion. The chlorpromazine was discontinued immediately and the patient was given 2,000,000 units of penicillin daily. The temperature persisted between 100° and 102°F. Two bone marrow aspirations and one bone marrow trephine biopsy showed only hypoplastic marrow.

After transfusion of one pint of whole blood, the blood count showed a hemoglobin of 9 gm. per 100 ml., an erythrocyte count of 3,000,000 per cu.mm., a leukocyte count of 600 per cu.mm., with 40 per cent segmented and 12 per cent nonsegmented polymorphonuclears. At this stage, the essential findings were persistent pyrexia, splenomegaly, anemia, and leukopenia associated with hypoplastic marrow. There was no evidence of the etiology of the underlying disease. The patient pursued a febrile, septic, progressively downhill course, and finally died twenty-five days following the onset of the septic fever.

Post-Mortem Findings

The body was that of a well-nourished white male. Autopsy of the head was not done.

Thoracic cavity: The mediastinum was markedly retracted to the left. The left main bronchus was surrounded by fibrous tissue and was somewhat stenosed. The left pleural cavity was obliterated by firm fibrous adhesions. The left lung was very small, atelectatic, and rather firm. The cut surface revealed many hard small nodules with a caseated center, surrounded by black lung tissue and connective tissue. The lesions were found predomi-

FIG. 2. Enlarged spleen as seen on an abdominal roentgenogram taken fifteen minutes after the patient swallowed 8 gm. of sodium bicarbonate and 200 ml. of orange juice.

nantly in the upper and middle parts of the lung. Some of the lesions were calcified. There was no evidence of active tuberculosis, either cavitary or miliary, on gross inspection. Several bronchi were dilated and filled with thick gray material.

The right lung was markedly enlarged. The division of lobes was abnormal, and no middle lobe could be identified. A diffuse emphysema was noted. The lung tissue was dry with minimal edema in the posterior half of both lobes. There was no evidence of either healed tuberculous lesions or of recent tuberculous dissemination.

Peritoneal cavity: There were numerous white nodules averaging 1 to 2 mm. in diameter, widely disseminated, especially in the upper one-half of the abdomen, covering the diaphragm, surrounding the liver, on the surface of the stomach, and in the major and minor omentum. Less frequently, they were encountered on the outer surface of the bowel. The lesions became less frequent near the pelvis. There was no free fluid in the peritoneal cavity.

Spleen: The spleen was considerably enlarged, weighing 470 gm. and measuring 20 cm.

in the largest diameter. Several easily freed adhesions were noted. There was no evidence of perisplenitis. The cut surface showed numerous, densely packed, miliary, soft, nodular lesions; most lesions were not in excess of 1 mm. in diameter. The largest were 8 mm. in diameter. Their form and size were quite suggestive grossly of Hodgkin's disease. The splenic pulp was somewhat pale, but the consistency was normal.

Liver: The liver was slightly enlarged. Only a few small nodules were seen through the capsule. The cut surface again showed a moderate number of the larger type of nodule similar to those described in the spleen. The lymph nodes at the hilus of the liver were greatly enlarged, the largest measuring 2 by 4 cm. These contained small nodular lesions similar to those in the spleen and liver. The same type of enlarged lymph node was encountered in the retroperitoneal tissues surrounding the upper part of the abdominal aorta. Pelvic lymph nodes were normal. The mucosa of the stomach and bowel was normal. The pancreas, adrenal glands, kidneys, and prostate were normal.

Histopathologic Findings

Microscopic examination of the spleen, liver, lymph nodes, and peritoneum showed multiple foci of necrosis surrounded by a very shallow rim of a few lymphocytes or histiocytes. The small lesions were not diagnostic of tuberculosis. However, the larger lesions showed necrosis with more typical caseation, surrounded in some areas by a few epithelioid cells and, in the liver, even by a few giant cells of Langhans' type. No Langhans' giant cells were encountered in the spleen and lymph nodes (figure 3).

Multiple sections of the left lung through the tuberculous lesions revealed old fibrocaseous tuberculosis of lung and bronchi. There was no histologic evidence of activity. Careful search revealed no evidence of miliary tuberculosis. The sections of the right lung revealed only emphysema.

Other findings included a mild fibrosis of the myocardium, mild atherosclerosis of aorta and coronary vessels, benign prostatic hypertrophy, and essentially normal kidneys, stomach, and bowel. The bone marrow gave the impression of being hypoplastic; no definite tuberculous lesions were seen.

Acid-fast stain of sections of the liver, spleen, and lymph nodes revealed chiefly a moderate number of tubercle bacilli in the lesions. However, some areas were teeming with acid-fast bacilli (figure 4). No tubercle bacilli could be found in the old pulmonary lesions

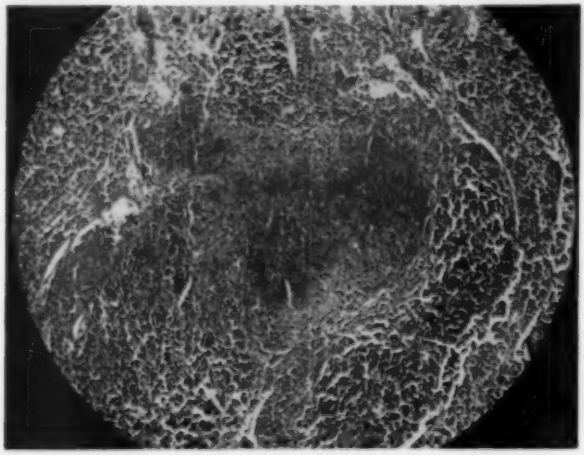

FIG. 3. One of many necrotic areas in a lymph node. Note absence of epithelioid and giant cells. (Hematoxylin and eosin, × 150)

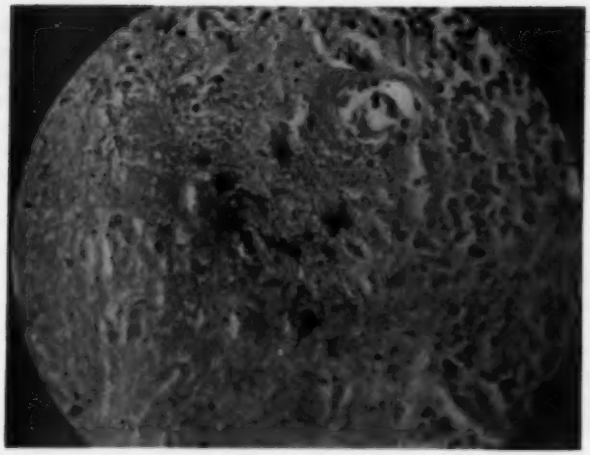

FIG. 4. Acid-fast bacilli in necrotic area of the abdominal lymph node. (Ziehl-Neelsen stain, × 660)

No evidence of extramedullary hematopoiesis was observed in the spleen, liver, or lymph nodes.

DISCUSSION

This case fulfills the clinical and pathologic criteria of so-called nonreactive tuberculosis, i.e., prolonged febrile course, splenomegaly, gross abnormality of hematopoiesis, disseminated necrosis, and an abundance of acid-fast bacilli in the lesions.

The patient had had chronic pulmonary tuberculosis for at least eight years. The disease was apparently brought under control by prolonged chemotherapy and bed rest over a period of several years. The chest roentgenogram became stationary about two years prior to death, and the sputum culture became negative for tubercle bacilli about twenty-two months prior to the onset of acute illness. After many years of satisfactory response to a tuberculous infection which apparently became well arrested, the body's defenses were suddenly overwhelmed in an unusual fashion.

Pathologic examination of the small and large bowels failed to reveal an ulcerative process that might be considered as a primary focus for extensive abdominal dissemination. Neither was there any evidence of tuberculosis in the mesenteric lymph nodes. The process of the spread must have been hematogenous. The right lung was surprisingly free of any gross tuberculous lesion. Scattered inconspicuous, minute foci may have been overlooked, although this is unlikely. The left lung revealed an old fibrocaseous tuberculous process of rather great extent; here again, there was no histologic evidence of activity. However, it is a well-known fact that careful search in old extensive lesions will often demonstrate small areas containing histologic evidence of relative activity.

It is reasonable to conclude that the old pulmonary lesion in the left lung should be considered as the probable source of dissemination. Rich (2) claimed that in each of his cases of acute caseating tuberculosis he could demonstrate a macroscopic focus from which bacilli were discharged into the blood stream. In most cases, the focus was a softened intimal lesion in a pulmonary vein. He explained the symtpoms as being due to a constitutional tuberculin reaction resulting from the intravascular discharge of large numbers of bacilli and necrotic material impregnated with tuberculoprotein in a hypersensitive

body. He also interpreted the swarms of bacilli in the lesions as being due to a considerable degree of local multiplication. It appears, therefore, that in nonreactive tuberculosis not only is there a sudden discharge of many bacilli into the circulation, but also there is a very definite depression of resistance that permits the free multiplication of the bacilli in the place in which they lodge.

Unfortunately, no specimen was obtained at autopsy in this case for thorough bacteriologic studies. Since this patient had had positive sputum cultures of typical human tubercle bacilli during the first five years of his illness, it can reasonably be assumed that his terminal illness was probably due to the same type of acid-fast bacteria. However, it has been observed that in the past few years many cases of tuberculosis-like diseases, with unusual clinical features caused by atypical strains of as yet unclassified acid-fast bacilli, have been reported (5–7).

Although the histologic appearance of nonreactive tuberculosis suggests an acute process, many of the patients who die with nonreactive tuberculosis have been ill for months. There is no definite evidence to indicate when the miliary tuberculous spread really began in the present case. The patient had a leukocyte count of 4,300 per cu.mm. sixteen months prior to the terminal event, and of 3,300 per cu.mm. about four months prior to death. Perhaps this slow, progressive leukopenia signified the insidious onset of miliary dissemination. On the other hand, the onset could well have coincided with the abrupt beginning of the febrile course.

It is generally believed that the changes in the blood picture of nonreactive tuberculosis are the result of the severe, acute, hematogenous spread of the tuberculosis. Arends (8), however, is in favor of the converse interpretation; he considers the nonreactive tuberculosis to be a result of a constitutional or an acquired insufficiency of the hemopoietic system, the abnormality of the blood predisposing to an abnormal tissue response to tuberculous infection. If his assumption is correct, in the present case the simultaneous administration of chlorpromazine and the development of an acute miliary spread suggest more than coincidence. The usual type of blood dyscrasia produced by chlorpromazine is agranulocytosis, but it is possible that it may also depress the bone marrow and produce a hypoplastic or aplastic type of anemia (9). The present writers are aware that there is no conclusive proof presented here to implicate chlorpromazine as the cause of the blood dyscrasia, but the history of exposure is inescapable. Possibly prolonged use of chlorpromazine in this case might have caused the anemia and leukopenia by depressing the bone marrow function, thus weakening the defense mechanism and enhancing the nonreactive type of tuberculous spread. Arends (8) reported one case of nonreactive tuberculosis which developed in a patient with agranulocytosis attributable to sulfadiazine therapy.

Rich and McKee (10) studied the acquired resistance to pneumococci. When immunized animals were deprived of their phagocytic leukocytes, they were completely unable to withstand pneumococcal infection even though the antibody titer remained unaltered. This showed that antibody protective power could be nullified by the leukopenia. Presumably, this is also true for tuberculous infection. According to Arend (8), Takeuhi (in Japan) has inoculated animals with tubercle bacilli after causing leukopenia by the administration of benzene and found that the animals showed very little resistance and died rapidly. The present writers point out that incrimination of the chlorpromazine in this case has been by inference and on the basis of association rather than on the basis of absolute proof.

From the clinical standpoint, the case presented was a difficult problem in diagnosis. The combination of splenomegaly, abnormal blood picture, and prolonged pyrexia were suggestive of some sort of obscure, primary hematologic disorder. Clinical ante-mortem

diagnosis of tuberculosis was made in a very few of the reported cases; in the majority, as in the present case, active tuberculosis was not suspected until it was demonstrated at autopsy. In view of the present chemotherapeutic agents available for the treatment of tuberculosis, it is essential that an early diagnosis be made. Therefore, it seems advisable that all cases of this sort be reported until it becomes generally recognized that tuberculosis should be considered either as a cause or as a complication in any undiagnosed case in which splenomegaly, an unusual blood count, and prolonged fever are found.

Summary

A case of nonreactive tuberculosis associated with anemia, leukopenia, splenomegaly, and pyrexia is presented. It developed in a patient with a treated and apparently healed tuberculous lesion in the lung.

The abnormal tissue response in this case and its possible association with chlorpromazine therapy is discussed.

Sumario

Tuberculosis Irreactiva: Presentación de un Caso

Preséntase un caso de tuberculosis irreactiva, acompañada de anemia, leucopenia, esplenomegalia y pirexia, que se produjo en un enfermo con una lesión tuberculosa en el pulmón, tratada y aparentemente curada.

Discútense la respuesta histológica anómala en este caso y su posible relación con la clorpromacinoterapia.

Resume

Tuberculose "non réactionnelle": observation d'un cas

Présentation d'un cas de tuberculose "non réactionnnelle" accompagnée d'anémie, de leucopénie, de splénomégalie et de pyrexie. L'affection s'est développée chez un malade porteur d'une lésion tuberculeuse pulmonaire traitée et paraissant cicatrisée.

La réponse tissulaire anormale, observée dans ce cas, et son association possible avec un traitement par la chlorpromaxine sont commentées.

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Notes

EFFECT OF CERTAIN AROMATIC AMINES ON SERUM ISONIAZID CONCENTRATIONS IN TUBERCULOUS PATIENTS[1]

Several investigators have shown that isoniazid is so rapidly and extensively metabolized by some humans that serum concentrations of biologically active drug may be very low or not measurable at all within a few hours after the oral administration of a test dose.[2-4] Serum isoniazid concentrations measured by the Kelley-Poet technique[5] (which measures total hydrazides, including free isoniazid, isoniazid hydrazones, and acetyl isoniazid) may be as high as 15 γ per ml., yet microbioassay[6] (which measures primarily free, unaltered isoniazid) of the same specimens indicates much lower concentrations of drug.[2, 3] Thus, significant amounts of isoniazid exist in the serum in biologically inactive forms.

Several metabolic products of isoniazid have been identified in the urine of humans following oral administration.[7] None of these metabolites, with the possible exception of isoniazid hydrazones,[8] possesses significant antimicrobial activity against *M. tuberculosis*.[9] Hughes[7] has shown that the most abundant of these metabolites, the acetylated form, may account for as much as 60 per cent of a test dose of isoniazid excreted in the urine.

Although it has not yet been demonstrated conclusively, preliminary observations indicate suboptimal therapeutic results in patients who metabolize isoniazid excessively.[10, 11] It might be anticipated that a better therapeutic response could be achieved if metabolic inactivation of isoniazid, especially acetylation, could be significantly inhibited.

Johnson[12] has shown that acetylation of isoniazid is effected by the same enzyme system as is acetylation of PAS, sulfonamides, and a large number of amino, amido, and imido compounds. More important, he demonstrated that competitive acetylation between any two of these drugs takes place both in pigeon liver homogenates and *in vivo* in rabbits. More recently, a significant increase in serum concentrations of biologically active isoniazid has been demonstrated in humans when PAS is administered simultaneously with isoniazid.[2, 3, 13]

[1] This study was aided in part by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, and also by a contract (No. SAph 67795) with the U. S. Public Health Service.

[2] MANDEL, W., *et al.*: Proc. Soc. Exper. Biol. & Med., 1956, *91*, 409.

[3] MORSE, W. C., *et al.*: Am. Pract., 1956, *7*, 1600.

[4] BELL, J. C., AND RIEMENSNIDER, D. K.: Am. Rev. Tuberc., 1957, *75*, 992.

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[9] BERNSTEIN, J., *et al.*: Am. Rev. Tuberc., 1952, *65*, 357.

[10] BIEHL, J. P.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 279.

[11] MITCHELL, R. S., AND BELL, J. C.: Unpublished data.

[12] JOHNSON, W. J.: Nature, 1954, *174*, 744.

[13] MORSE, W. C., *et al.*: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis Veterans Administration, Army, and Navy, 1956, p. 283.

The purpose of the present investigation was to apply further those observations in humans by administering isoniazid with each of several aromatic amines which are metabolized by acetylation and which might therefore reduce the rate of isoniazid acetylation.

Serum concentrations of biologically active isoniazid were measured by microbiologic assay in a group of patients after isoniazid was given alone, and again after isoniazid was given simultaneously with each of the aromatic amines tested, i.e., PAS, para-aminobenzoic acid (PABA), sulfanilamide, and sulfadiazine.

At least two premises are fundamental to these metabolic studies: (*1*) the reliability of the microbioassay method used to measure serum concentrations of isoniazid, and (*2*) the constancy of the pattern of isoniazid metabolism in any individual as reflected by serum isoniazid concentrations determined by that method. The validity of each of these premises has been previously established.[4, 6]

Twenty tuberculous persons were selected at random at the Colorado General Hospital. Preceding the days on which the test protocols were carried out, all antituberculous chemotherapy except streptomycin was discontinued at least fourteen hours before the first test dose was given; usually this period was forty-eight hours or more, and in several instances the patient had never been given antituberculous medication. An interval of at least twenty-four hours elapsed between the administration of each successive test dose of drug or combination of drugs.

The testing procedure was as follows: Breakfast was omitted. Each test dose of drug, or combination of drugs, was given in a single oral dose on an empty stomach at 7:00 a.m. A fat-free breakfast, consisting of dry toast and jelly, with 180 ml. of skimmed milk, fruit juice, or black coffee, was given at 8:30 a.m. Blood specimens were drawn at two, three, and six hours after drug administration. After the first few patients were investigated, it became apparent that there was usually little difference in the serum concentration at two and three hours; therefore, the three-hour blood concentrations were abandoned for the sake of expediency and convenience.

The test dose of isoniazid was 4 mg. per kg. of body weight; of PAS, 4 gm. of the acid or 5.57 gm. of the sodium salt; of sulfanilamide, 0.9–1.5 gm.; of sulfadiazine, 1.0–1.5 gm.; and of PABA, 3 or 4 gm.—all by mouth.

The method used for estimating serum concentrations of biologically active isoniazid has been reported previously.[6] The results are presented in table 1.

Previous studies have demonstrated that on repeated testing with isoniazid alone, serum concentrations of biologically active drug followed a reproducible pattern for any individual.[6, 14, 15] Therefore, the serum isoniazid concentration obtained when a subject was given isoniazid alone served for comparison with serum bioassay values obtained after the same subject had been given isoniazid plus some other drug. If the repeated test yielded the same value as the first test, it was recorded as "0" (no variation); if it yielded a value one tube higher, i.e., if the end point of growth inhibition increased from 0.8 to 1.6 γ per ml., or from 1.6 to 3.2 γ per ml., et cetera, it was recorded as + 1 (variation of one tube greater). Conversely, if the repeated run yielded a value one tube lower than

[14] Morse, W. C.: Personal communication.

[15] Middlebrook, G.: Personal communication.

TABLE 1

THE EFFECT OF AROMATIC AMINES ON SERUM ISONIAZID CONCENTRATIONS

Patient	Time	Serum Bioassay Concentration of Isoniazid (γ/ml.)				
		Isoniazid Alone	Isoniazid and PAS	Isoniazid and PABA	Isoniazid and Sulfadiazine	Isoniazid and Sulfanilamide
	hours					
1. Mi	2	1.6	3.2	3.2	3.2	1.6*
	3	1.6	3.2	3.2	—	1.6
	6	0.8	1.6	1.6	1.6	0.8
2. Ja	2	1.6	3.2	6.4		3.2
	3	—	—	—		—
	6	0.8	1.6	1.6		1.6
3. Wa	2	1.6	6.4	3.2	3.2	3.2*
	3	1.6	3.2	3.2	—	3.2
	6	0.8	1.6	1.6	1.6	1.6
4. He	2	1.6	3.2	3.2	3.2†	
	3	1.6	3.2	3.2	3.2	
	6	0.8	1.6	3.2	1.6	
5. Ra	2	0.8	3.2	3.2‡	3.2	
	3	0.8	1.6	3.2	3.2	
	6	0.8	0.8	1.6	1.6	
6. Li	2	3.2	3.2	3.2	1.6	3.2*
	3	1.6	3.2	3.2	—	3.2
	6	0.8	3.2	3.2	0.8	3.2
7. Di	2	1.6	1.6	1.6		
	3	1.6	1.6	1.6		
	6	0.8	1.6	1.6		
8. Da	2	3.2	3.2	3.2		
	3	—	—	—		
	6	0.4	1.6	1.6		
9. Ma	2	1.6	1.6	3.2‡	3.2	
	3	—	1.6	3.2	3.2	
	6	0.4	0.8	1.6	0.8	
10. (*a*). Ea	2	0.8	0.8	3.2‡	1.6	1.6
	3	0.4	0.8	1.6	0.8	—
	6	0.4	0.4	0.8	0.4	0.2
10. (*b*). Ea	2	3.2	1.6	3.2		
	3	—	—	—		
	6	0.4	0.8	1.6		
10. (*c*). Ea	2	3.2		6.4		
	3	—		—		
	6	0.4		1.6		
11. Do	2	1.6	1.6	1.6‡	1.6	0.8§
	3	0.8	1.6	1.6	0.4	—
	6	0.2	0.4	0.8	0.2	0.2
12. Ba	2	0.8	3.2	1.6		1.6
	3	—	—	—		—
	6	0.2	0.4	0.8		0.2

TABLE 1—*Continued*

Patient	Time	Serum Bioassay Concentration of Isoniazid (γ/ml.)				
		Isoniazid Alone	Isoniazid and PAS	Isoniazid and PABA	Isoniazid and Sulfadiazine	Isoniazid and Sulfanilamide
	hours					
13. Ar	2	1.6	3.2	3.2		1.6
	3	—	—	—		—
	6	<0.2	0.4	0.8		0.2
14. Co	2	0.4	1.6	1.6		1.6
	3	0.2	—	—		—
	6	0.2	0.2	0.4		0.2
15. Mo	2	3.2	1.6	1.6		0.8
	3	—	—	—		—
	6	0.2	0.2	0.4		0.2
16. Me	2	0.4	1.6	3.2	0.8	1.6
	3	—	1.6	—	0.4	—
	6	<0.2	1.6	0.4	0.2	0.4
17. Mar	2	0.8	3.2	3.2		0.8
	3	—	—	—		—
	6	0.2	0.4	0.4		0.2
18. Hen	2	1.6	3.2	3.2		
	3	—	—	—		
	6	0.4	0.8	1.6		
19. Bu	2	3.2	1.6	3.2		
	3	—	—	—		
	6	<0.2	<0.2	<0.2		
20. La	2	0.8	0.8	0.8		
	3	—	—	—		
	6	<0.2	<0.2	0.4		

* Sulfanilamide, 0.9 gm. dose; all other patients were given 1.5 gm. dose.
† Sulfadiazine, 1.5 gm. dose; all other patients were given 1 gm.
‡ PABA, 3 gm. dose; all other patients were given 4 gm. of PABA.
§ Sulfanilamide, 1.33 gm. dose; all other patients were given 1.5 gm. dose.

the first test, it was recorded as −1 (variation of one tube lower). The same procedure was followed for differences of two and three tubes. These results are presented graphically in figure 1.

The upper graphs in figure 1 demonstrate the reproducibility of serum isoniazid concentrations as measured by bioassay on repeated testing of any individual given isoniazid alone. This information was derived from a group of thirty persons and has been described more fully in a previous report.[6] The middle and lower graphs represent the maximal variations obtained in the group of 20 subjects in this study by taking serum isoniazid concentrations after isoniazid alone as the reference for comparison with values obtained after the simultaneous administration of isoniazid and an aromatic amine, i.e., isoniazid-PAS or isoniazid-PABA. Results were recorded in this manner in an effort to minimize changes in serum bioassay concentrations which may be more apparent than real.

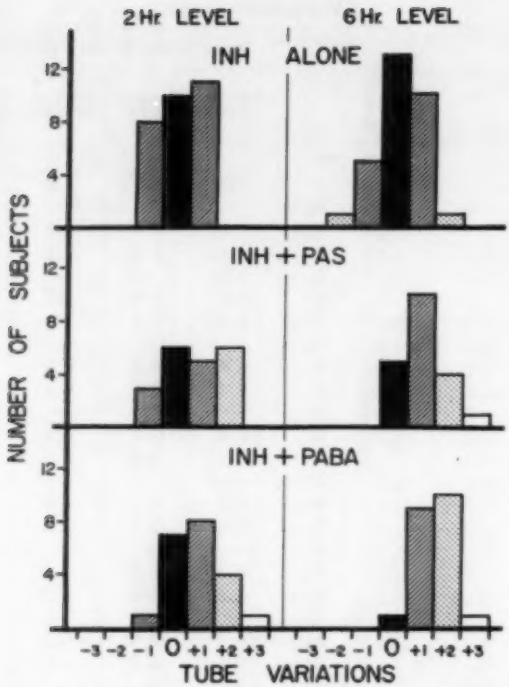


FIG. 1. Serum levels of biologically active isoniazid obtained two and six hours after testing with isoniazid alone, with isoniazid-PAS, and with isoniazid-PABA. Upper graphs represent the maximal variation in bioassay results obtained on repeated testing with isoniazid alone in a group of 30 subjects. The bioassay result obtained on initial testing was taken as a reference point for comparison with all subsequent bioassay determinations. The middle and lower graphs represent maximal variations in bioassay results obtained in a group of 20 tuberculous patients; results after isoniazid alone were used as reference points for comparison with results obtained after the simultaneous administration of isoniazid and PAS (middle graphs) and isoniazid and PABA (lower graphs).

It should be recognized that the increases in bioassay concentrations are not expressed in equal increments, i.e., an increase from 0.8 to 1.6 γ per ml. or from 1.6 to 3.2 γ per ml., et cetera, represents a difference of only one tube in the level of growth inhibition. Of equal importance is that reproducibility of serum concentrations on repeated testing may vary rarely as much as two tubes; this variation is apparently governed by chance (upper graphs of figure 1). Assuming that the same principles of reproducibility apply to the second group of patients (middle and lower graphs, figure 1), it is immediately apparent that the addition of PAS or PABA to orally administered isoniazid increases serum concentrations of biologically active drug.

Since PABA or its metabolites might possess some inhibitory effect upon the

strain of tubercle bacilli used for bioassay, tubes containing serial dilutions of 2 to 400 γ of PABA per ml. of medium were inoculated in the same manner as in the test. No inhibitory effect was noted. Sera from 4 humans given 4 gm. of PABA without other drugs, and subjected to the standard testing procedure, showed no antimicrobial activity against the test strain of tubercle bacilli used in the bioassay procedure. Similar control measures have been performed with PAS.[13-15]

Three features concerning the effect of administering these compounds with isoniazid should be noted: (*1*) the serum concentration of isoniazid usually was both elevated and prolonged; (*2*) whereas the two-hour concentration of isoniazid was not always increased, it sometimes was maintained over a longer period than when isoniazid was given alone; and (*3*) in spite of the obvious effect in most subjects, certain patients (*Nos. 11, 15, 19,* and *20,* table 1) failed to show an increase in the serum concentration of biologically active drug.

The effect of PABA is somewhat surprising because this compound is reportedly metabolized in humans predominantly by conjugation with glycine and glucuronic acid, only very small amounts being acetylated.[16] However, some workers have reported significant acetylation of PABA in humans[17] and, probably of equal importance, some of the conjugates of PABA such as para-aminohippuric acid are acetylated.[18] A delay in acetylation of PABA, being preceded by its conjugation with glycine, may account for some of the prolonged effect observed with that drug.

The response of isoniazid concentrations to the sulfonamides was similar, but less constant and often less marked than with PAS or PABA. Considering the doses of sulfonamides employed, this may be due to inadequate "overloading" of the acetylating capacity.

* * *

It seems reasonable to suspect that some treatment failures with isoniazid may be due, in part at least, to extensive metabolic degradation of this compound by some patients. It may also be suspected that some of the benefits derived from isoniazid-PAS therapy are not due entirely to delayed emergence of bacterial resistance or to the antimicrobial activity of PAS, but partly to competitive metabolism. It seems equally plausible to anticipate an improved response to isoniazid therapy in selected patients if they are given PABA or some other aromatic amine in addition to isoniazid. Other compounds, which are similarly metabolized,[19] such as PAS amide, might be more useful clinically and are being investigated at this time.

It should be emphasized that these observations were made on serum isoniazid concentrations and indicate only that the concentrations of biologically active drug are often increased by the addition of these other compounds. They neither

[16] Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics, ed. 2, The Macmillan Co., New York, 1955, p. 1715.

[17] Gershberg, H., and Kuhl, W. J., Jr.: J. Clin. Investigation, 1950, *29*2, 1625.

[18] Tabor, C. W., *et al.*: J. Pharmacol. & Exper. Therap., 1951, *102*, 98.

[19] Johnson, W., and Corte, G.: Proc. Soc. Exper. Biol. & Med., 1956, *92*, 446.

confirm nor refute the tentative explanation that this is the result of reduced acetylation.

Preliminary observations of the urinary excretion of isoniazid and isoniazid metabolites on these patients indicate that the addition of these compounds inhibits not only acetylation but also the further metabolism of isoniazid to isonicotinic acid and other conjugates. Moreover, there is strong evidence which indicates that as one metabolic pathway is blocked, secondary alternative pathways may be used to a major extent.

Because of the wide variation of metabolic pattern one wonders about the advisability of determining isoniazid dosage, not on the basis of mg. per kg. of body weight as is currently done, but rather in accordance with the degree to which each individual patient metabolically inactivates the drug.

In summary: Serum concentrations of biologically active isoniazid were often increased by the simultaneous administration of PAS, PABA, sulfanilamide, or sulfadiazine with isoniazid to a group of tuberculous patients. This effect is explained tentatively on the basis of competitive acetylation, but not all subjects exhibited such a response and there is some evidence which indicates the utilization of alternative metabolic pathways. It is suggested that isoniazid be administered according to each individual's metabolic pattern, rather than on the basis of body weight.

The Colorado Foundation for Research in Tuberculosis and
The Department of Medicine, University of Colorado School of Medicine
Denver, Colorado

J. Carroll Bell
D. K. Riemensnider
Roger S. Mitchell

January 4, 1957

Correspondence

THE ABSORPTION OF SHELLAC-COATED PAS GRANULES WITH SPECIAL REFERENCE TO THE AGE OF THE PREPARATIONS

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

At a meeting of the Danish Association of phthisiologists, Professor K. A. Jensen in his lecture on the treatment of tuberculosis warned against the employment of unduly resistant coating of PAS granules as this may impede absorption of the active substance. The concentration in the blood may in this way remain below the therapeutic level. Attention has also been drawn to this condition by other investigators.[1]

In Spangsbjerg Sanatorium, Esbjerg, Denmark, determinations of para-aminosalicylic acid in the blood in patients following the administration of various PAS preparations were undertaken. In the determination of free para-aminosalicylic acid in the blood, Professor Lehmann's[2] colorimetric micro-method was employed using Pulfrich's photometer. Following administration of a single dose of 4 gm. of various shellac-coated PAS granules with the same content of para-aminosalicylic acid, very varying blood concentrations were obtained in the same patient under otherwise identical experimental conditions. With a preparation which was 3½ years old, blood concentration values of less than 2 mg. per 100 ml. only were obtained in 3 patients and another portion of the same origin which was 1½ years old rendered blood concentrations of 6–7 mg. per 100 ml. in the same patients.

On investigation of the time of disintegration in artificial "intestinal juice," a distinct prolongation of the time of disintegration could be demonstrated, related to the age of the preparation. The portions mentioned had originally a time of disintegration of 11 and 15 minutes, respectively, but when employed 3½ and 1½ years later, the time of disintegration was increased to >60 and 30 minutes, respectively. Investigation of other shellac-coated PAS granules which had been stored for long periods, and the time of disintegration of which had previously been determined, rendered corresponding results.

The employment of shellac-coated PAS granules stored for long periods is deprecated in the treatment of tuberculosis as the effect of treatment with such preparations must be regarded as dubious.

THE NATIONAL ASSOCIATION FOR THE FIGHT
AGAINST TUBERCULOSIS
SPANGSBJERG SANATORIUM
ESBJERG, DENMARK

Yours sincerely,
CURT E. TARNOWSKI

February 26, 1957

RAPID MICROCULTURE METHOD FOR THE ISOLATION OF TUBERCLE BACILLI

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

A detail of technique which we have found important in obtaining early growth of microcultures (Hambleton, A., and Ozols, J.: Am. Rev. Tuberc., 1957, *75*, 1007) is to use

[1] SANZ, M. C., AND MÜLLER, A.: Schweiz. med. Wchnschr., 1952, *82*, 1334.
[2] LEHMANN, J.: Scandinav. J. Clin. Lab. Investigation, 1951, *3*, 306; 1952, *4*, 142.

potassium hydroxide instead of sodium hydroxide for the digestion of sputum samples and to neutralize with citric acid, or a citric acid-phosphoric acid mixture, instead of hydrochloric acid. Without this modification, about three more days are required before the microcultures are positive.

The reason for this is that potassium citrate or phosphate is found in almost all synthetic culture media for tubercle bacilli. In contrast, the rather high concentration of sodium chloride, which is an end product of the usual sodium hydroxide-hydrochloric acid method, is definitely unfavorable to tubercle bacilli.

THE BECK MEMORIAL SANATORIUM
LONDON, ONTARIO
May 3, 1957

Sincerely yours,
ARTHUR HAMBLETON
J. OZOLS

Books

THERAPY OF FUNGUS DISEASES. Edited by Thomas H. Sternberg and Victor D. Newcomer. Pp. 337 with illustrations, Little, Brown and Company, Boston, 1955. Price $7.50.

The 56 papers in this volume were presented at an International Symposium to stimulate the exchange of ideas on the therapy of fungal diseases. The committee at the University of California in Los Angeles and the Squibb Institute for Medical Research are to be congratulated on the choice of scientists as contributors. The general articles are particularly stimulating, especially that of Selman Waksman, who provides a compact summary of antifungal antibiotics and their biologic properties and chemistry. The contributions of Ajello, of Lubarsky, and of Egeberg *et al.* on the distribution of pathogenic fungi in the soil contribute to our knowledge of epidemiology. Dr. Emmons suggests that the distribution of *Cryptococcus neoformans* in pigeon droppings merits its consideration as a possible etiologic factor in some groups of cases.

A group of dermatologists and mycologists from abroad furnished data about the incidence and types of mycoses. In India, France, and the Philippine Islands, the dermatomycoses were emphasized, and in these and other countries the varying incidence of etiologic agents was described. Histoplasmosis was recognized comparatively frequently in Argentina and in Mexico, and in French Africa. Coccidioidomycosis was seen in Argentina and Mexico. Actinomycosis was seen in France and Argentina and nocardiosis due to *N. braziliensis* in Mexico. Sporotrichosis was noted to have disappeared from the practice of mycologists in Paris, but was seen in fairly large numbers in Mexico. Simuangco from the Philippine Islands cast a critical eye on case reports and emphasized the need for thorough identification and comparison of etiologic agents isolated from patients.

The volume will be most useful because of the presentation of current data about antifungal therapeutic agents. Some of these are well established in the armamentarium, such as sulfur for plant mycoses, and the aromatic diamidines for North American blastomycosis; others are new. The *in vitro* and *in vivo* antifungal action and toxicity of a long list of substances is reported; cinnamic acid, nitrostyrene, filipin, sulfonamides and sulfones, chloroquinaldol, antibiotic ✱1968 (Nepara), rhodanine, and actidione. All of these are either ineffective in one or more tests or toxic. Mycostatin is described in 16 papers. This drug is highly active against many pathogenic fungi *in vitro*, but is relatively insoluble in water and is quite toxic. Because it is not absorbed from the gastrointestinal tract it is used for topical and parenteral therapy. Cutaneous and gastrointestinal infection due to *Candida albicans* responds favorably and promptly to therapy with mycostatin. Several authors reported on the therapy of systemic *Histoplasma capsulatum* and *Coccidioides immitis* infections with mycostatin. The results in man were not striking, and its use is associated with disturbing local pain and systemic reactions.

Other therapeutic agents for histoplasmosis are ethyl vanillate, beta-di-ethyl-aminoethyl-fencholate, hydroxystilbamidine, aminostilbamidine, for cases of disease in man, and sulfonamides in laboratory experiments. Other therapeutic agents for coccidioidomycosis were aminostilbamidine for cases of infection in man, and antihistamines in laboratory experiments. The therapy of mycetoma with a sulfone and with D-25, or 2′2-dioxy-5′5-achlor-diphenyl sulfide were described as promising. The volume demonstrates the extent and depth of activity in the search for antifungal therapeutic agents and their clinical testing and will be especially valuable to research workers in that field.

W. D. Sutliff, M.D.
Memphis, Tennessee

Pathologic Physiology, Second edition. Edited by William A. Sodeman, The W. B. Saunders Company, Philadelphia, 1956.

This is the second edition of a text that has enjoyed wide popularity for six years. The volume is the collaborative product of twenty-nine highly respected physicians. Its common theme is a treatise on disease as it manifests itself in derangements of the normal physiologic processes. Throughout the text, the principles of the basic sciences, notably those of physiology and, to a lesser degree, those of biochemistry and pathology, are used to clarify the too often stereotyped precepts of medicine. Recognition of the didactic value of such an approach is clearly reflected in the current trend of medical education toward "vertical teaching" programs.

The stated aim of the second edition is to keep pace with recent advances in medicine. This aim has been accomplished only in part. Many chapters, notably those pertaining to the pancreas, the blood and spleen, the joints, the lungs, and the gastrointestinal tract remain essentially unaltered by recent advances in these fields. Worth-while revisions and additions have been made in sections dealing with the kidney, the liver, and the heart. Several new chapters have been added. One entitled "Genetics of Abnormal Growth and Neoplasia" is provocative but contains many highly controversial and speculative, if not erroneous, concepts. A chapter on the nervous system treats this subject in generalities and in so doing loses much of its practical value in the broad scope of the subject material.

Most physicians have already learned to make frequent use of *Pathologic Physiology* and, although the revisions of the second edition are not extensive, this volume should be readily available to all those whose practice of medicine engages the fundamental concepts of physiology.

F. Stephen Vogel
New York, New York

Current Therapy 1956. Latest Approved Methods of Treatment for the Practicing Physician. Edited by Howard F. Conn. Pp. 632, W. B. Saunders Company, Philadelphia, 1956. Price, $11.00.

Current Therapy and other similar annual review books help students and practitioners inform themselves of the clinical implications of the innumerable

clinical drug trials and current comparative evaluations of these and other forms of medical and surgical treatment. The benefits and hazards of current treatments and the potential uses of newer methods under study are best known to scholars and practitioners active in specific fields of medicine. The panels of consultants and contributors assembled by Conn for this volume have provided useful information of this type.

Books like *Current Therapy* supplement rather than supplant standard texts. These, similar to encyclopedic textbooks for medicine and surgery, require about three years to prepare, in part because extended periods are required for evaluation and testing of ideas and procedures. This prolonged period is necessary for an adequate trial and comparative evaluation of new drugs with the more than two thousand pharmaceutical products currently available and under examination.

The present 632 page book contains fifteen sections devoted to infectious disease, diseases of various organ systems, and diseases due to physical and chemical agents. A roster of drugs and conventional tables of weight and measure forms a concluding chapter. Subject and author indices are included. The printing is clear and well spaced in double columns.

The contributors have stated and justified their recommendations clearly. Controversy is at a minimum. However, there are a few places where the use of the term "approved methods" in the title has implied incorrectly that controversy has been resolved. It is not generally accepted that antimicrobial drugs are of benefit in primary atypical pneumonia; tetracycline drugs are not often the drugs of choice in bacterial meningitis; intrathecal use of antimicrobials need not be harmful; there is little scientific justification for the use of the "stress fortified" antimicrobial products.

Other details suggest that improvements can be made in the editing of this book. The section on relapsing fever does not include reference to the causative agent, *Borrelia recurrentis*; there is repeated use of the terms "bacteriostasis" and "bactericidal activity," which are relative terms, both applicable to a single antimicrobial drug, depending upon the experimental conditions employed. The section on tuberculosis extends over ten pages and could be abbreviated considerably. The streptococci with penicillin tolerances in excess of one microgram per milliliter discussed in the section on endocarditis probably are of the enterococcus variety rather than *Streptococcus viridans*.

The generally consistent use of statements of the aim of treatment of various diseases, the results which can reasonably be anticipated, and the indications for specialist consultation provide useful background for the usually concise one-two-three order of recommended procedure and drug treatment. This text will be useful to those whose libraries do not include current journals or recently published textbooks.

CARL BERNTSEN
New York, New York

American Trudeau Society

Medical Section of the National Tuberculosis Association

Report of the Thirteenth Pembine Conference

The thirteenth Pembine Therapy Conference was held on September 7, 8, and 9, 1956, at the Four Seasons Club, Pembine, Wisconsin. Fifty conferees were present from Michigan, Minnesota, and Wisconsin. These were selected by each state and represented men most active in the field of thoracic diseases. Eleven guests were invited by the Pembine Conference Committee:

Leroy Berard—Chicago, Illinois
Hiram Langston—Chicago, Illinois
George L. Loeb, San Haven, North Dakota
David F. Loewen—Decatur, Illinois—also guest presentor.
James D. Murphy—Baltimore, Maryland
Paul Murphy—St. Louis, Missouri
Edward L. Ross—Winnipeg, Manitoba
John D. Steele—San Fernando, California
Joseph B. Stocklen—Cleveland, Ohio
Julius L. Wilson—Philadelphia, Pennsylvania
Frank W. Webster—New York, New York

A short report of the proceedings may be of interest to those who have never been to this unique Conference, which freely admits that it is the oldest and best in our country. For those who were present, there may be less interest because each will feel that he could write a better review. This attitude is peculiar to the Pembine Conference where each conferee and guest feels that in some aspect of thoracic diseases he is THE specialist—and often he is.

The general topic was Thirty Consecutive Sanatorium Admissions immediately prior to January 1, 1956. The object of the Committee in picking this date was to get as recent a summary of tuberculosis therapy as possible.

The case reviews from the tri-state area were presented by:

Morgan Heights Sanatorium, Marquette, Michigan
Middle River Sanatorium, Hawthorne, Wisconsin
Minnesota State Sanatorium, Ah-Gwah Ching, Minnesota

The guest presenting hospital was:

Macon County Tuberculosis Sanatorium, Decatur, Illinois

These sanatoriums have facilities for less than 100 patients each, except the Minnesota State Sanatorium. The medical director of each hospital presented the cases and was held responsible for all therapy, including surgery except the actual technique of the operations. He had personal knowledge of each patient and had to assume the responsibility for the treatment. There were no "staff decisions," which experience has shown to be so often merely a screen to hide behind, after an unfortunate—or even unwise—method of treatment had been used.

True to form, the first case presented provoked considerable criticism concerning the necessity for surgical resection in minimal lesions which have done well on chemotherapy and could be presumed to do well on prolonged chemotherapy. Most—but by no means all—of the conferees agreed that some tuberculosis does heal to such an extent that it is safer to leave it in the lung than to resect it. There was a vociferous minority—at Pembine there is always a vociferous minority—which believed that resection is indicated

even when the remnants are small, hard in appearance, and have never produced tubercle bacilli which could be cultured from the sputum.

Thirteen Pembine Conferences have taught us that the vociferous minority of today may represent the accepted view of tomorrow. A case in point is the early opponents of pneumothorax, who were right and still are. There were also in the dim past minorities favoring phrenic nerve operations and pneumoperitoneum—and these have now been converted.

There also developed a loud, but not widespread, demand for bacterial confirmation of every diagnosis of tuberculosis. This would lead logically to carrying out Koch's four postulates on many minimal cases, with the addition of diagnostic thoracotomy. Without previous chemotherapy, this can be a rather hazardous procedure if the lesion is tuberculous.

There is more widespread recognition each year of other pulmonary diseases which simulate tuberculosis and the Conference is becoming increasingly demanding of bacteriologic or pathologic proof. This places an added load on the laboratory and its personnel. Histoplasmosis has already assumed a major place in the differential diagnosis of any pulmonary lesion, as was well illustrated by the cases from Illinois.

Surgery had the floor for one session when the medical men had an opportunity to review 18 surgical emergencies of the chest, other than of the heart. These were presented by:

William Tuttle, Detroit, Michigan; Nathan K. Jensen, Minneapolis, Minnesota; and Paul F. Hausemann, Milwaukee, Wisconsin.

For the men who do not do thoracic surgery, this was probably one of the most unusual sessions since the inception of Pembine. The weird and bizarre were represented by an auto accident in which the gear shift lever penetrated the thorax to the depth of the aorta and spine. It was successfully removed, of course, by a delayed thoracotomy and that surgeon now rates very high with the police and accident department of his city.

This had to be topped by the next presentor. A case of emergency tracheotomy done with a rusty pen knife by the surgeon on a colleague at a formal banquet, both in evening clothes, probably saved a life. In memory, some were carried back to the days when formal attire in the operating room was as common as the present day's colorless and sterile gowns.

Herniation of the diaphragm by the intestines or liver seemed to be the most common emergency and occurred at any age. It can usually be repaired through the thorax by pushing or the abdomen by pulling. The important point is to make the diagnosis; the "pushers" or the "pullers" can repair the damage very skillfully, they said.

A unanimity of opinion seldom reached at this Conference was attained on the advisability of delaying surgery as long as feasible in any case—this could be from five minutes to a year. The object is to operate when the patient, and probably also the surgeon, reaches his best condition.

Suggestions for improving the Conference included more uniform presentation of data, fewer cases, more discussion, and more formalization of procedure. These were received with thanks by the Conference Committee. If any one thinks that a Pembine Conference can be formalized to any degree, he just hasn't been to a Conference. Its uniqueness and leadership in this field is due to its planned lack of formalization and its rugged individualism.

The next Conference will be held September 6, 7, and 8, 1957.

Committee for the Pembine Conference

SUMNER COHEN, *General Chairman*
RICHARD P. JAHN
EVERETT W. GAIKEMA
MORRIS THOMAS, *Secretary*

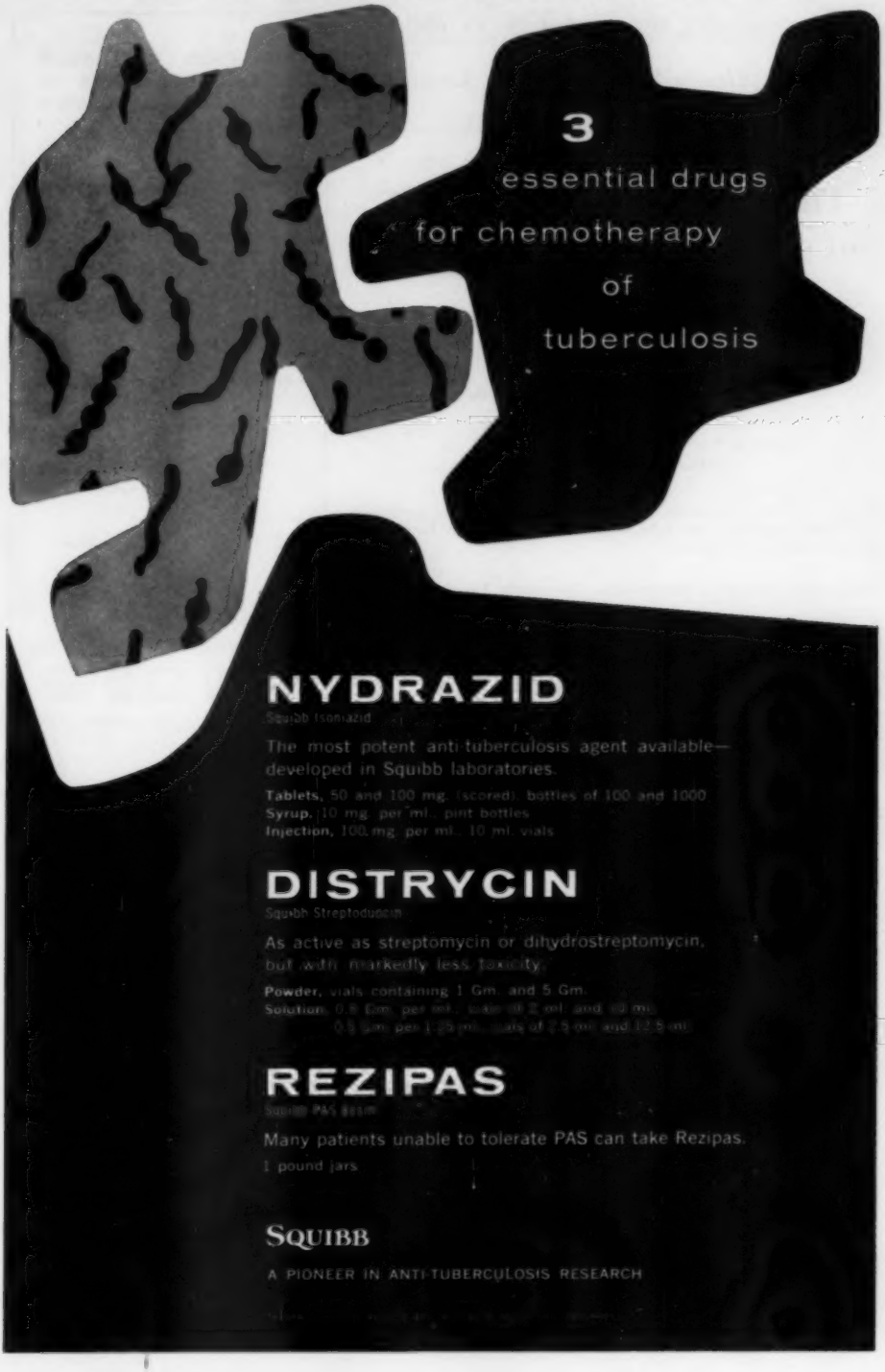
NOTICES

The following date and location of the next annual meeting of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 19–24

INCREASED MEMBERSHIP DUES AND SUBSCRIPTION RATE TO THE REVIEW

On recommendation of the Council of the American Trudeau Society, the membership present at the annual business meeting of the Society on Tuesday, May 7, 1957, in Kansas City, Missouri, voted to increase the annual membership dues to $18 and subscription to THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES to $15, effective January 1, 1958. This increase becomes necessary because there has been a 46 per cent increase in the cost of production of the REVIEW since 1954.


3
essential drugs
for chemotherapy
of
tuberculosis
NYDRAZID
Squibb Isoniazid
The most potent anti-tuberculosis agent available—developed in Squibb laboratories.
Tablets, 50 and 100 mg. (scored), bottles of 100 and 1000
Syrup, 10 mg. per ml., pint bottles
Injection, 100 mg. per ml., 10 ml. vials
DISTRYCIN
Squibb Streptodusoin
As active as streptomycin or dihydrostreptomycin, but with markedly less toxicity.
Powder, vials containing 1 Gm. and 5 Gm.
Solution, 0.5 Gm. per ml., vials of 2 ml. and 10 ml.
0.5 Gm. per 1.25 ml., vials of 2.5 ml. and 12.5 ml.
REZIPAS
Squibb PAS Resin
Many patients unable to tolerate PAS can take Rezipas.
1 pound jars
SQUIBB
A PIONEER IN ANTI-TUBERCULOSIS RESEARCH

Announcing the First Title in the

John Alexander Monograph Series

The Surgical Management of Pulmonary Tuberculosis

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JOHN D. STEELE, M.D.

Introduction by
FREDERICK A. COLLER, M.D.

Biographical Sketch of John Alexander by
CAMERON HAIGHT, M.D.

Written for both surgeons and internists by thoracic surgeons who were trained by the late John Alexander and by two of his close medical associates.

Covers all Phases of Procedures Currently Used:

- Indications
- Techniques
- Results
- Standard and plombage thoracoplasty
- Treatment of tuberculous empyema
- Cavernostomy

John C. Jones (Los Angeles) writes on **pulmonary resection for tuberculosis in the pre-chemotherapy era,** including his own experiences in the 1930's; Max Chamberlain (New York) on **segmental resection;** Herbert Sloan and Roger Milnes (Ann Arbor) on the **indications, contraindications, and results of resection.** Problems and methods of the reduction in the size of the pleural cavity with pulmonary resection are discussed in a chapter on combined collapse and resection.

Some of the historical aspects of the surgery of pulmonary tuberculosis have been drawn from John Alexander's books published in 1925 and 1937 and from the uncompleted manuscript for the third book on which he was working at the time of his death.

A short chapter on the surgical management of tuberculosis in the mentally ill has been included since this is an important subject and little discussed.

Since **chemotherapy** plays such an indispensable role in the medical and surgical treatment of pulmonary tuberculosis, a comprehensive chapter (which is actually a short monograph) on the subject has been included.

This chapter by Winthrop N. Davey (Ann Arbor) and John B. Barnwell (Washington, D. C.) contains historical material as well as practical suggestions for modern treatment. The topics covered include details on antimicrobial agents (both the antibiotics and synthetics), the selection of patients, the selection of drugs, a review of the results and limitations of drug therapy, and the use of chemotherapy in extrapulmonary tuberculosis.

Publication date: May 15, 1957 **248 pages (7" × 10")**

175 illustrations **Cloth $9.50**

THE JOHN ALEXANDER MONOGRAPH SERIES on Various Phases of Thoracic Surgery

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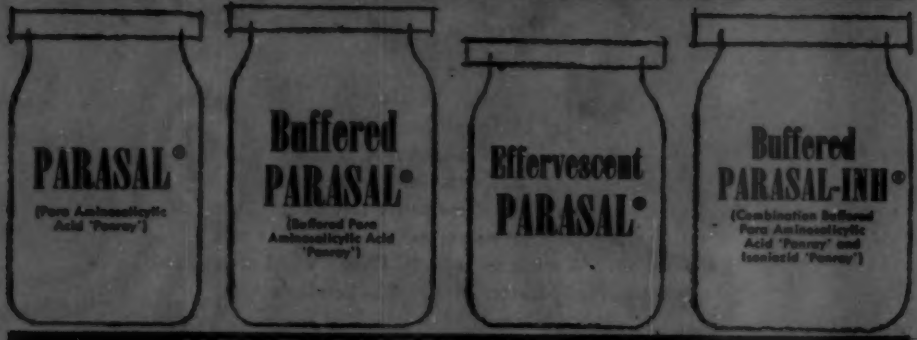

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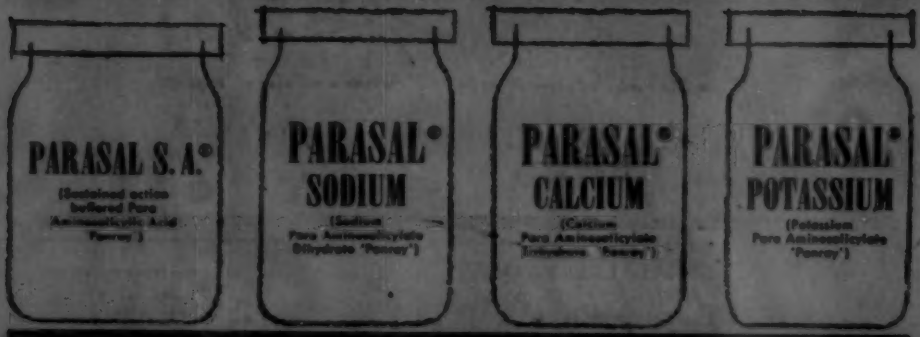

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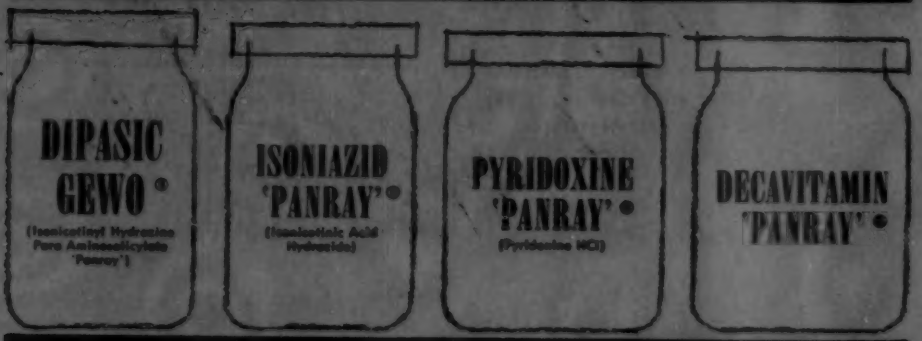

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